Newborn Screening (NBS) programs are a vital public health resource, ensuring millions of infants have the opportunity to benefit from early detection and intervention of genetic, endocrine, metabolic, hearing, and critical congenital heart conditions. The Innovations in Newborn Screening Interoperability (INBSI) Resource Center is a HRSA-sponsored project, established to assist in addressing the gaps and barriers in the current NBS data exchange eco-system with the aim of enhancing NBS data interoperability. Through this effort, the INBSI team has documented past, current, and future NBS interoperability initiatives across the country. Over the past 18 months, INBSI has worked directly with multiple state and jurisdiction level newborn screening programs and have provided assistance in documenting existing and planned processes for electronic data exchange. This gives INBSI a unique perspective on the NBS landscape and how interoperability has influenced these surveillance and follow-up programs.

In this poster we will describe various interoperability initiatives and the challenges encountered prior, during, and following implementation. From initial data collection and message transport to internal processing and data ingestion, state programs have tackled interoperability in different ways. Variables such as state size, population, geography, and local policies are obvious factors in how these processes are performed, but what does that really mean with regard to interoperability implementations? HL7 v2 messaging may be the answer for some state programs, but maybe not for others. This poster will outline various data sharing methods, what challenges were encountered during implementation, what is being done currently to promote interoperability, and what electronic data exchange may look like in the future.

**Presenter:** Craig Newman, Altarum, craig.newman@altarum.org
The Potential for Newborn Screening to Transform Disease Understanding Through Data Retention and Sharing

A. Brower, K. Chan, J. Taylor, G. Tona, Y. Unnikumaran and L. Barnes, American College of Medical Genetics and Genomics, Bethesda, MD

Each year almost 4 million newborns are screened for treatable conditions using both physiological and blood-based methods. This routine testing generates millions of data points representing a wide variety of biological processes at a unique, neonatal time point. These data points could transform disease understanding, both for conditions that are part of, and candidates for, newborn screening (NBS). However, less than half of the 53 newborn screening (NBS) programs retain residual dried blood samples beyond one year, and there is incomplete information about the retention of digital data points resulting from laboratory and hospital-based assessments. This is a missed opportunity to advance disease understanding because the unselected cohort of newborns screened in the United States and around the world reflects the racial, geographic, economic, and educational diversity of our planet. In fact, this may be the perfect cohort to transform disease understanding because although every newborn receives essentially the same screen, other factors vary including access to treatment and a known family history of disease. Many of the screened conditions have comorbidities, including intellectual disabilities, and these children receive a variety of interventions that could be tracked and analyzed to identify critical periods of development and intervention. The Newborn Screening Translational Research Network (NBSTRN) has created data tools and resources, along with data retention and sharing policies, for use in NBS research. Collectively, this has resulted in longitudinal phenotypic data for sixty-eight conditions being available for secondary research. We will describe the data portfolio contained in the Longitudinal Pediatric Data Resource (LPDR) and propose approaches used in the LPDR that could be implemented during NBS screening. The goal is to retain, harvest, and repurpose the millions of data points generated during routine screening to help transform disease understanding.

**Presenter:** Amy Brower, American College of Medical Genetics and Genomics, abrower@acmg.net
ELSI Advantage: A Resource for the NBS Community to Facilitate Inclusion of ELSI in Newborn Screening Research

C. Lumpkins¹, K. Chan¹, E. Goldman², A. Goldenberg³, I. Holm⁴, A. Brower¹; ¹American College of Medical Genetics and Genomics, Bethesda, MD, ²University of Michigan, Lansing, MI, ³Case Western Reserve University, Cleveland, OH, ⁴Harvard Medical School, Boston, MA

Introduction: Each year in the United States, 3.8 million newborns are screened for up to 81 genetic conditions, and over 12,000 are diagnosed and referred to clinical care. Discoveries of novel technologies to screen, diagnose, and treat genetic diseases have led to an ever-increasing list of conditions that are candidates for newborn screening (NBS). The NBS community of researchers, parents and families, advocacy groups, healthcare professionals, and state NBS programs play important roles in the research that leads to these advancements. Because NBS research often involves and impacts newborns and children, consideration of the ethical, legal, and social issues (ELSI) is crucial during the planning, execution, and reporting of NBS research. To facilitate the inclusion of ELSI in NBS research, we describe efforts to create a web-based resource called ELSI Advantage.

Methods: The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Hunter Kelly Newborn Screening Research Program funds the Newborn Screening Translational Research Network (NBSTRN) operated by the American College of Medical Genetics and Genomics (ACMG). NBSTRN creates data tools and resources and operates expert workgroups to support the NBS research community. A literature and key effort review was conducted to identify topic areas and the Bioethics and Legal Workgroup informed the design of ELSI Advantage, a resource for the NBS community.

Results: To address the broad range of ELSI concerns across stakeholder groups, ELSI Advantage is organized in several sub-tools: Ask ELSA!, NBS ELSI 101, Research Repository, Policy Map, and Expert Workgroup Consultations. NBS ELSI 101 provides an introductory overview of the key ELSI concerns of each NBSTRN stakeholder group—researchers and healthcare professionals, families and advocacy groups, and state NBS programs. The Research Repository is a curated resource that directs researchers and other interested stakeholders to key publications addressing ELSI in NBS research. The Policy Map facilitates collaboration between state NBS programs and researchers by providing national and state-level views of state NBS program policies and procedures that impact research. ELSI Advantage also allows researchers with specific questions about ELSI and their research project to request expert consultations with an ELSI expert. Ask ELSA! is an interactive tool that allows users to ask questions about ELSI and NBS Research. Ask ELSA! draws from a curated database and identifies relevant information within ELSI Advantage.

Conclusion: To ensure NBS research both maximizes benefits and mitigates potential harms, researchers ought to consider the ELSI of newborn screening and NBS research during each stage of their research project. The NBSTRN’s ELSI Advantage aids the newborn screening research community in considering ELSI in their research.

Presenter: Caroline Lumpkins, American College of Medical Genetics and Genomics, clumpkins@acmg.net
Data Science and Autism: Exploring the Use of Newborn Screening Data to Understand Genetics and Clinical Outcomes
Z. Talebizadeh, C. Lumpkins and A. Brower, American College of Medical Genetics and Genomics, Bethesda, MD

Introduction: Newborn screening (NBS) is the largest public health genetic program with 3.8 million newborns screened for up to 81 genetic conditions each year. Many of these conditions have comorbidities, including autism, intellectual and/or developmental disabilities (IDD), and these comorbidities impact health outcomes across the lifespan. AutGO (Autism Genetics Outcomes), is an initiative to support broad stakeholder partnerships and promote a new integrated concept called GO. GO is a research approach that draws on both genetics and clinical outcomes perspectives. We also developed an engagement protocol for collecting stakeholders’ feedback to inform development of GO hypotheses. These efforts have built a foundation for developing a targeted data science capacity to promote conducting patient-centered outcomes research (PCOR) on mental health related conditions.

Methods: A diverse range of perspectives is necessary for co-producing research related to heterogeneous conditions such as autism/IDD. Understanding the mechanisms underlying variable expressivity of core behavioral symptoms and responsiveness to treatments is a critical step toward informing personalized approaches to treatment of these complex conditions. The substantial investments made by the research community and participating patients in developing repositories that archive de-identified clinical and research data, provide an unprecedented opportunity to leverage these resources for understanding clinical outcomes. However, such resources were not designed for this purpose, posing significant challenges to harnessing data from existing repositories to conduct PCOR. Addressing these challenges requires strategic scaffolding to integrate the knowledge and perspectives from both scientific silos and the populations impacted by mental health conditions. Due to the comprehensive nature of the program, data obtained from NBS has the potential to improve population health in an equitable manner. Using a mixed methods approach (community-based participatory approach, systematic reviews, and qualitative methods) we will assess NBS tools and resources for data science capacity pertaining to mental health outcomes.

Results: We will describe a roadmap for conducting PCOR in autism/IDD using data science approaches that include: 1) applicable NBS tools and resources, and 2) identified barriers, facilitators, and motivators, along with 3) suggested recommendations to overcome existing challenges.

Conclusion: Findings from this capacity building project will stimulate interest within the autism research community to consider utilizing NBS data and resources in developing longitudinal studies. The renewed awareness of the risk of autism/IDD after a positive NBS and diagnosis could lead to improving treatment guidelines for mental health conditions.

Presenter: Zohreh Talebizadeh, American College of Medical Genetics and Genomics, ztalebizadeh@acmg.net
Celebrating a Decade of Conversations to Facilitate Newborn Screening Pilots: NBSTRN National NBS Pilot Monthly Webinar

J. Taylor, K. Chan and A. Brower, American College of Medical Genetics and Genomics, Bethesda, MD

Newborn screening (NBS) began over sixty years ago with screening for one condition, phenylketonuria (PKU). Today, newborns in the United States are screened for up to 81 conditions, with sixty-one comprising a Recommended Uniform Screening Panel (RUSP). States determine which conditions to screen but the RUSP, established by a federal advisory committee, informs the makeup of state panels. The Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) also established a nomination and evidence review process that is open to all stakeholders. A key component of a nomination is the completion of a prospective pilot in an unselected population of newborns. NBS pilots test the analytical and clinical validation of novel technologies to screen and diagnose conditions. The duration of some pilots are also long enough to establish the net benefit of early intervention and treatment. The first condition that was approved by this committee was severe combined immunodeficiency (SCID), and the addition of SCID continues to inform the expansion of NBS in important ways, including the use of the Newborn Screening Translational Research Network (NBSTRN). To advance NBS research, the NBSTRN develops data tools and resources, operates expert workgroups, and coordinates events to foster discussion among the NBS community. One of these efforts was to coordinate a SCID screening pilot in high and low birth number states using the T-cell receptor excision circle (TREC) assay, the first molecular assay used as the primary screening method. To provide a forum for discussion and information sharing, the NBSTRN began hosting the National NBS Pilot Monthly Webinar in 2011, with an initial focus on SCID, followed by other conditions that were recently nominated or recommended to the RUSP. These web-based meetings have been well attended with an average of ~80 stakeholders representing over 50% of states, and provide a platform for researchers, state NBS programs, clinicians and advocates to share information, screening algorithms, pilot results and clinical findings.

A decade later, NBSTRN continues to host this webinar each month to facilitate information sharing between the NBS community about conditions recently added to the RUSP or conditions that are parts of pilots. Since the initial webinars in 2011, the scope of the conditions discussed has expanded to include seven other conditions as well as presentations on topics of interest in the NBS community. The conditions were selected based on the multiple pilot studies funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), the Centers for Disease Control (CDC), and others. This presentation will provide an overview of the past decade of discussions and highlight trends shared from across the NBS community.

Presenter: Jennifer Taylor, American College of Medical Genetics and Genomics, jtaylor@acmg.net
What Does Long-Term Follow-Up Mean to You?: A Discussion with State NBS Programs

J. Taylor¹, J. Baysinger², C. Johnson³, A. Burke⁴, J. Hauser⁵, J.A. Bolick⁶, K. Chan¹, L. Barnes¹, Y. Unnikumaran¹, A. Bower¹; ¹American College of Medical Genetics and Genomics, Bethesda, MD, Oklahoma State Department of Health, ²Oklahoma City, OK, ³Iowa Newborn Screening Program, Iowa City, IA, ⁴North Dakota Department of Health, Pierre, ND, ⁵Minnesota Department of Health, St. Paul, MN, ⁶Arkansas Children’s Hospital, Little Rock, AR

Problem and Objective: Over 20,000 newborns are diagnosed each year with a congenital condition through the newborn screening (NBS) system. The majority of these conditions require life-long care and management, ideally with the care coordinated through a medical home, to assure the best possible outcomes for each diagnosed baby. All stakeholders in the NBS community (parents, patients, clinicians, researchers, and NBS programs) play important roles in long-term follow-up (LTFU), but there is no national system of LTFU data collection, analysis, sharing, and reporting. In addition, LTFU activities, policies, and practices vary across state NBS programs. The goal of this roundtable discussion is to provide state NBS programs a forum to discuss their involvement in LTFU and to create a series of definitions of LTFU for each stakeholder group. A new initiative will also be described to capitalize on clinical care efforts to deliver LTFU into a centralized resource that will improve the insight of the benefits of NBS.

Methodology: The roundtable will include three short presentations. The first two presentations will be from states with established LTFU. A third presentation will be from the American College of Medical Genetics and Genomics (ACMG) to describe the LTFU-Cares and LTFU-Checks Initiative, a LTFU model system with Spinal Muscular Atrophy (SMA) as a test case. Then the group will break into teams for further conversation on what LTFU activities are currently feasible in their state and to draft a definition of LTFU that matches current and planned efforts. Each participant will be provided with a worksheet with a working definition of LTFU for public health programs and they will identify what LTFU currently looks like in their state, discuss how their program is currently fulfilling these activities, and next steps to strengthen their program or a baseline for starting a LTFU program.

Results/Conclusion: Newborn screening is aims to assure the best possible outcomes for newborns identified with disorders that benefit from early identification and intervention. The LTFU of diagnosed newborns is an important component and this roundtable will result in a better understanding of what LTFU means for state NBS programs, and will begin to outline recommended activities that could be included in a LTFU program.

Presenter: Kee Chan, American College of Medical Genetics and Genomics, kchan@acmg.net
Early Functional Vision Screening in the Neonatal Intensive Care Unit: What Can It Tell Us?
R. King¹, C. Smyth²; ¹Children’s Eye Physicians, Centennial, CO, ²Anchor Center for Blind Children, Fort Collins, CO

Standard of care addressing vision concerns for infants in the Neonatal Intensive Care Unit (NICU) is focused on retinopathy of prematurity (ROP) only. However, premature infants are also at increased risk for brain-based visual impairment that retrospectively is diagnosed in older children and is quickly becoming a public health concern in the United States according to the National Institutes of Health. We also know that early brain plasticity means we can support infants through early intervention, so identifying the risk of visual concerns in the NICU and during the first year of life can improve their long-term outcomes. This year Anchor Center for Blind Children, a non-profit that supports families with young children with visual impairment, and a NICU at Rocky Mountain Hospital for Children in Denver, Colorado, has collaborated to complete a research project that identified visual function concerns using a validated screening instrument, the Neonatal Assessment Visual European Grid (NAVEG). Our objective was to determine if the use of this screening instrument for neurological risk can be successfully administered in the NICU and if it potentially detected early cases of brain-based visual impairment.

This pilot validation study was approved to screen up to 130 infants in the NICU of all babies born at 31 6/7 weeks or before or those identified and referred by the neonatologist. These infants were already identified to need ROP examinations but did not necessarily have active ROP. The NAVEG screening was administered by two research credentialed non-medical personnel that were trained by the pediatric ophthalmologist who acted as the primary investigator for the study.

Forty-nine participants withdrew from the study due to early discharge or aging out. Eighty-one successful screenings led to 26 (32%) referrals for follow-up ophthalmological care. Screenings were conducted on 49 male and 32 female infants at an average age of 37.8 weeks. The average age of gestation was 27 weeks.

Statistical results on the screening validation included an acceptable Cronbach’s alpha of .74 scale reliability. Every item was determined to contribute to the screening except for Eye Abnormalities, which confirms our hypothesis that the NAVEG screening addresses the need for identification of brain-based visual impairment. At this time, family follow-up data to determine the positive and negative predictive values are still being collected. Only five infants have been diagnosed with delayed visual maturation indicating possible false positives. Studies in additional NICUs are planned to collect needed scores for validation.

This pilot study indicates that the NAVEG is a credible screening tool to administer in the NICU with infants from 35 to 40 weeks to identify early brain-based visual concerns.

Presenter: Catherine Smyth and Robert King, Anchor Center for Blind Children,
csmyth@anchorcenter.org

APHL 2022 Newborn Screening Symposium – October 16-20, 2022 – Tacoma, WA
California Newborn Screening – Validation of NeoBaseTM2 Assay on QSightTM Systems

**Background:** Newborn genetic disorders such as organic acidemias, aminoacidopathies, purine metabolism disorder, tyrosinemia type-1, and X-linked adrenoleukodystrophy (X-ALD) are screened by measuring abnormal levels of analytes such as amino acids, acylcarnitines, succinylacetone, adenosine, and 26:0-lysophosphatidylcholine in dried blood spots (DBSs) using tandem mass (MSMS) spectrometry. Recently the NeoBaseTM (NB) kit used for California MSMS screening was replaced with the NeoBaseTM 2 (NB2) kit from PerkinElmer Inc. During implementation of NB2, new MSMS instruments, QSightTM 210 MD (QS), were also introduced. Several thousand archived specimens and multi-levels of controls were analyzed to establish various analytical and clinical specifications.

**Methods:** The NB2 kit measures 51 different analytes in a single assay in the presence of 29 different internal standards (IS). The analytes are extracted from a 3.2mm DBS using 125μL extraction solution with IS and hydrazine in a 96 well plate by incubating for 30min at 45°C. 100μL of the extract is transferred and incubated for 1hr at 27°C. 10μL of the DBS extract is injected into QS instrument for measurement in the MRM mode. SimplicityTM software acquires and processes the QS data. For the validation, a 20-day study was performed using system and multilevel controls. A method comparison between NB2 with NB assays (on TQD, Waters Inc.) was done by analyzing 4200 newborn specimens. In addition, 1500 specimens were measured separately for C26:0-LPC (X-ALD) evaluation.

**Results:** The low and high system controls showed an average imprecision (CV%) of 8.72, and 7.68, with an accuracy of 12.89% and 15.34%, respectively. For low and high controls, 95% of analytes differed by less than 20% from the analyte target values assigned by the vendor. The multilevel (L1-L6) spiked samples showed a linear correlation coefficient of 0.99-1.0 with an average imprecision of 7%. The average difference of means and medians for analytes in patient specimens were around 5%, signifying an even statistical distribution for setting measurable ranges. The average analyte recoveries for the assay were 85%. A comparison of NB2 to the NB nonderivatized method showed a correlation of 0.99-1 for all analytes for the newborn screening range.

**Conclusion:** The NeoBase 2 kit is validated for newborn screening at the Genetic Disease Laboratory, and five different regional labs, on a total of thirteen QS systems. The overall imprecisions and recoveries are within acceptable limits. The QC ranges established for the controls provide measurements within quality specifications for each analyte. Post-implementation challenges pertaining to glutaric acidemia type-I and X-ALD measurements were overcome by retuning of instruments and introducing a wash step respectively. The presumptive positive rate for NBS disorders was within acceptable limits.

**Presenter:** Kumaran Ramanathan, California Department of Health, kumaran.ramanathan@cdph.ca.gov
Pompe Disease Newborn Screening in California After Four Years


**Background:** Pompe disease (glycogen storage disease type II) is a rare autosomal recessive lysosomal storage disorder caused by a deficiency in the acid-α-glucosidase (GAA) enzyme. Low GAA enzyme levels lead to accumulation of lysosomal glycogen, particularly in the heart and skeletal muscles. Pompe disease is clinically heterogeneous and can be categorized into three types: classic infantile-onset Pompe disease (IOPD) (with cardiac involvement), non-classic IOPD (without cardiac involvement), and late-onset Pompe disease (LOPD). Newborn screening (NBS) for Pompe disease in California (CA) started on August 29, 2018. We describe CA’s Pompe disease screening algorithm and outcomes, variants identified, and clinical follow-up findings after four years of screening.

**Methods:** Pompe disease NBS in CA is a two-tiered process. In tier-1, flow injection analysis-tandem mass spectrometry (FIA-MS/MS) measures GAA enzyme activity in dried blood spots. Specimens with low GAA enzyme activity (< 10% of the daily enzyme activity median) are immediately called out as screen positive and referred to one of 15 metabolic Special Care Centers (SCCs) for clinical follow-up and confirmatory testing. All specimens with enzyme levels ≤18% of the enzyme activity median undergo tier-2 DNA sequencing to identify variants in the GAA gene. If at least one pathogenic variant or variant of uncertain significance (VUS) is found, cases are considered screen positive and referred to a SCC. SCCs report the findings from diagnostic evaluation for all referrals and treatment information when indicated. Annual long-term follow-up (LTFU) reports are provided by SCCs for confirmed cases through age 5 years.

**Results:** As of March 1, 2022*, 1,545,100 newborns were screened for Pompe disease. 173 (0.011%) were screen positive and referred to a SCC for follow-up. Four cases were resolved as classic IOPD, 35 as LOPD, and 13 as not-otherwise-specified (NOS), for an overall Pompe disease birth prevalence of 1/29,713. DNA sequencing identified 101 unique GAA variants among a total of 328 variants, with 43% identified as pathogenic alleles, 40% as pseudodeficiency alleles, and 17% as VUS. 3 of 4 infants diagnosed with IOPD had cardiac and other symptoms and all received treatment with enzyme replacement therapy, one of which expired due to a choking episode. One newborn diagnosed with IOPD remains asymptomatic without treatment at three weeks of age. 39 patients diagnosed as LOPD or NOS are actively being followed in LTFU and nearly all are asymptomatic.

**Discussion:** In the first four years of Pompe disease NBS in CA, the birth prevalence was within the range of previously reported estimates. Less than half of the GAA variants identified were known pathogenic variants. LTFU will be critical for better understanding the clinical significance of these cases.

*Updated data will be provided to include four full years.

**Presenter:** Jamie Matteson, California Department of Public Health, jamie.matteson@cdph.ca.gov

---

*Updated data will be provided to include four full years.*

**Presenter:** Jamie Matteson, California Department of Public Health, jamie.matteson@cdph.ca.gov
An Epidemiological Approach to Setting and Monitoring Cutoffs for a New Mass Spectrometry Screening Assay

J. Matteson, H. Tang, K. Ramanathan, P. Neogi, T. Bishops and S. Sciortino, California Department of Public Health, Richmond, CA

**Background:** On January 27, 2022, the California Newborn Screening (NBS) Program transitioned to the new NeoBase™ 2 non-derivatized MS/MS kit from the old PerkinElmer NeoBase™ non-derivatized MS/MS kit. This report describes the epidemiological process used to evaluate analytes measured on the new MS/MS kit, set new cutoffs, and monitor screening outcomes.

**Methods:** Prior to implementation, we measured all analytes for 3,956 banked specimens in parallel on the old and new MS/MS kits, including 531 specimens from infants confirmed to have a disorder detectable by NBS. We also measured C24:0 LPC and C26:0 LPC (newly available in NeoBase™ 2) for 2,084 additional banked specimens, including 123 specimens from males diagnosed with X-linked adrenoleukodystrophy. We summarized the distribution of the analyte values on both kits for confirmed and unaffected cases. We assessed whether if implemented, the new MS/MS kit would miss confirmed cases with the historical cutoff, or there would be an increase in false positives, and therefore evaluated whether a new cutoff would be suitable. We ran a linear regression between the values measured on the old and new MS/MS kits, and when there was good correlation, we predicted the values for the new MS/MS kit in a 6-month sample of the screening population and for all confirmed cases. We then evaluated several cutoff scenarios based on percentiles and z-scores in which we tabulated the number of missed cases and false positives in the validation sample, missed cases in the confirmed registry cases, and false positives in the 6-month screening sample. Cutoffs were chosen to minimize the number of false negatives and to keep the positives at historical rates. To monitor screening data in near-real-time, we created Tableau® dashboards that connect to our screening information system database and refresh nightly.

**Results:** Cutoffs for 24 out of 54 analytes measured by MS/MS testing were modified. After implementation, two analytes caused considerable spikes in positives, which were detected immediately using our monitoring dashboards. Machine tuning modifications corrected the issue with one analyte and a cutoff modification corrected the issue with the second. Our MS/MS screen positive rate increased after implementation from 22 in 10,000 to 33 in 10,000. No false negatives have been identified in 2 months since implementation.

**Conclusions:** Epidemiological techniques proved to be valuable for setting cutoffs for our new screening assay. In addition, near-real-time monitoring of screening data after implementation was critical in mitigating issues as soon as they arose. While our screen positive rate increased after implementation, no false negatives were observed. Further evaluation of cutoffs will be useful in bringing positive rates down to historical rates or lower once more population screening data is available.

**Presenter:** Jamie Matteson, California Department of Public Health, jamie.matteson@cdph.ca.gov
Freeze/Thaw Stability of 17OHP, T4, TGal and TSH Analytes in Dried Blood Spots
C. Brown, G. Pena, E. McCown, J. Mei and P. Petritis, Centers for Disease Control and Prevention, Atlanta, GA

Background: The Newborn Screening and Molecular Biology Branch (NSMBB) at the CDC manufactures and distributes dried blood spot (DBS) quality assurance (QA) materials that resemble newborn specimens. These specimens are used for quality control (QC), validation of new screening tests, and proficiency testing (PT). NSMBB is accredited to the International Organization for Standardization and the International Electrotechnical Commission (ISO/IEC) 17043 standard for PT Providers. Key performance criteria for quality assurance materials includes an understanding of the environmental impact on biochemical analyte stability in the DBS matrix characterized by an evaluation of analyte recovery during storage conditions. As such, a freeze/thaw (F/T) study was conducted to determine whether DBS QA materials were fit for testing under extreme conditions.

Method: The effects of continuous freeze/thaw cycles were individually measured on the following four analytes: thyroxine (T4), thyroid stimulating hormone (TSH), 17-hydroxy progesterone (17OHP), and total galactose (TGal). Sets of single, highly enriched DBS specimens were stored in low gas permeable bags with and without desiccant and placed at -20°C and 4°C. The DBS were allowed to reach room temperature then placed back at -20°C and 4°C respectively for 10 cycles with at least one day in between each cycle. For every F/T cycle, a pair of DBS was removed from the set and stored at -20°C until the end of the study. Analyte recovery was determined by testing DBS specimens from each cycle in a single analytic run.

Results: Analyte recovery was evaluated for DBS undergoing up to 10 F/T cycles and stored with desiccant at -20°C. Percent (%) difference for each analyte ranged from 5-14% for 17OHP, 3-12% for T4, 1-10% for TGal, and 1-10% for TSH. There were no significant differences between F/T cycles for the analytes when DBS were stored with or without desiccant within a temperature series, either -20°C or 4°C (p < 0.05). There was also no difference when the first and tenth F/T cycles were compared directly. The recovery of the 17OHP stored with desiccant showed a significant difference per cycle (10 F/T) when the -20°C and 4°C temperature series were compared. There were no significant differences for the other analytes.

Conclusions: For most of the DBS analytes assessed in this study, T-test analysis did not show a significant difference and null hypothesis was true, demonstrating their stability for up to 10 F/T cycles when stored at -20°C and 4°C with or without desiccant. The exception was 17OHP, for which there was a significant difference when stored at 4°C with desiccant compared to -20°C with desiccant. T4, TSH, and TGal are stable in DBS for at least two weeks when stored at -20°C or 4°C, even with daily F/T cycles and without desiccant. For longer term storage, it is recommended that DBS specimens be stored at -20°C with desiccant for optimal stability.

Presenter: Christofer Brown, Centers for Disease Control and Prevention, qhk3@cdc.gov
Multiplexing Iduronate 2-Sulphatase (for MPSII) with the LSD 6-Plex Assay Using Cold-Induced Aqueous Acetonitrile Phase Separation
E. Courtney, A. Pickens, C. Cuthbert and K. Petritis, Centers for Disease Control and Prevention, Atlanta, GA

Introduction: The Recommended Uniform Screening Panel (RUSP) recently added the Lysosomal Storage Disorder (LSD) mucopolysaccharidosis type II (MPS II, Hunter Syndrome) to be included for routine screening. There is a subsequent need to multiplex and improve screening for the MPSII Iduronate 2-Sulphatase (I2S) enzyme activity into current methodologies that screen 6 additional LSDs (6-plex). Structural similarity between substrates for I2S and alpha-L-iduronidase (IDUA) result in cross reactivity of the I2S substrate with IDUA, making it difficult to create a true 7-plex assay. Therefore, current methods focus on I2S as a stand-alone assay (1-plex), and extracts from 6-plex and 1-plex assay are combined downstream after enzymes are inhibited. This study investigates cold-induced aqueous acetonitrile phase separation (CIPS) to improve the combination of 6-plex and 1-plex extracts, which increased method throughput and signal of all LSD 7-plex products.

Methods: 3.1 mm punches of quality control (QC) dried blood spot materials and neat solutions were used in the analysis. The 6-plex and 1-plex assay were incubated in separate plates, then quenched. Extracts were combined to form the 7-plex assay. Sample clean-up was performed using brine and ethyl acetate solutions or CIPS using acetonitrile (ACN). ACN was chilled to -20°C prior to addition, then samples were sealed, centrifuged, and placed in -20°C freezer for 10 min. CIPS with ACN produced a biphasic layer allowing for removal of the upper ACN layer containing the LSD enzyme products. Sample extracts were dried, resuspended in mobile phase, then analyzed using LC-MS/MS in throughputs similar to other newborn screening methods.

Results: Using CIPS with acetonitrile results in improved detection for all analytes, without requiring additional chemical steps. This method results in more complete coagulation and sedimentation of heme and proteins extracted from the DBS than centrifugation alone, therefore producing cleaner samples for LC-MS/MS analysis. The signal for I2S was higher with more resolved chromatographic peaks using the CIPS method than with acetonitrile alone, and other analytes did not appear to be adversely affected. Using CIPS for analyte extraction from DBS appears promising and straightforward for achieving cleaner sample extracts in a new, 7-plex LSD screening panel. To our knowledge, this is the first time CIPS has been used to process DBS specimens for newborn screening applications.

The findings and conclusions in this study are those of the authors and do not necessarily represent the official position of the U.S. Department of Health and Human Services, or the U.S. Centers for Disease Control and Prevention (Division of Laboratory Sciences).

Presenter: Elya Courtney, Centers for Disease Control and Prevention, pli3@cdc.gov
Development of Spinal Muscular Atrophy Proficiency Testing and Quality Control Programs
C. Greene, K. Greene, A. McCabe, F. Lee, S. Cordovado and C. Cuthbert, Centers for Disease Control and Prevention, Atlanta, GA

Spinal muscular atrophy (SMA) is a genetic neuromuscular condition that results in the progressive loss of muscle strength over time and affects the ability to sit up, walk, and in severe cases, breathe, and swallow. SMA is an autosomal recessive condition with approximately 95% of cases caused by common deletions of exon 7 in the survival motor neuron 1 (SMN1) gene. Laboratory screening to detect babies at risk for developing SMA is based on measuring the presence or absence of exon 7 in the SMN1 gene.

Newborn screening for SMA in the United States began with four programs in 2018 and after SMA was added to the Recommended Uniform Screening Panel in 2019, the adoption of screening has rapidly expanded to include nearly all U.S. newborn screening programs by early 2022. The Centers for Disease Control and Prevention’s Newborn Screening Quality Assurance Program (CDC NSQAP) first provided pilot SMA proficiency test (PT) materials for domestic screening programs in 2020 and routine PT in 2021. In 2022, NSQAP expanded enrollment in the SMA PT program to include international newborn screening programs. Since the SMA PT program launch, NSQAP has shipped over 6000 dried blood spots (DBS) to 86 states or countries to ensure accurate testing for this disease. The next steps to support SMA newborn screening is the development of a quality control program also based on detecting the presence or absence of exon 7 of the SMN1 gene. All CDC DBS materials for SMA screening are created from lymphocytes isolated from SMA patients or carrier parents recruited by the Sequoia Foundation who contracts with participating SMA clinics. Donated lymphocytes were transduced by CDC using the Epstein Barr Virus, immortalizing the cells to create a sustainable source of SMA material. These materials are essential in supporting the large-scale creation of DBS for quality assurance. To support comprehensive quality assurance of SMA including proficiency testing and newborn screening quality control, CDC developed novel procedures to grow massive quantities of transduced cells using tissue culture bioreactors. Transduced cells were combined with leukodepleted blood and serum to mimic newborn DBS. This material has been used with great success both in the U.S. and now internationally. In addition, future expansions to the SMA quality assurance program includes characterizing all repository samples for SMN2 copy number, which impacts the severity of disease. CDC’s SMA sample repository currently consists of 30 patient/family samples (25 confirmed cases and 5 family carriers) which will be assessed for SMN2 copy number. Since SMN2 copy number testing is being used as second tier test by some newborn screening programs, these materials can be used for quality assurance by those labs.

Presenter: Chris Greene, Centers for Disease Control and Prevention, crg0@cdc.gov
The NBS Molecular Training Workshop
L. Hancock¹, C. Saavedra-Matiz², R. Lee³, G. Zarbian³, O. Akinsola⁴, J. Ojodu⁴, C. Cuthbert¹, S. Cordovado¹; ¹Centers for Disease Control and Prevention, Atlanta, GA, ²Wadsworth Center, New York State Department of Health, Albany, NY, ³Texas Department of State Health Services, Austin, TX, ⁴Association of Public Health Laboratories, Silver Spring, MD

There has been an increasing use of molecular assays by newborn screening (NBS) laboratories over the years, creating a need for molecular laboratory training support specific to NBS. Since 2010, the Association of Public Health Laboratories (APHL) and CDC’s Newborn Screening and Biology and Molecular Biology Branch (NSMBB) have co-sponsored the NBS Molecular Training Workshop which is held annually and is free to all participants.

The NBS Molecular Training Workshop was established subsequent to the cystic fibrosis (CF) screening workshops which began in 2008. The CF workshops were attended by both laboratory and follow-up participants and focused on clinical outcomes, follow-up requirements and laboratory techniques particularly for CF molecular testing. Three CF workshops were held within screening public health laboratories in Austin, TX, Madison, WI, and Jamaica Plain, MA.

In 2010, the CF workshop was broadened in scope to include all molecular testing in NBS and moved to CDC’s NSMBB laboratories. Over the years, the workshop lecture topics have expanded to highlight new disorders, new or disruptive technologies (multiplex genotyping platforms, and next generation sequencing) as well as special laboratory considerations unique to molecular testing (unidirectional workflow, molecular laboratory design, molecular assay validation, and variant interpretation). The workshop involves both lectures and hands on laboratory work. In the early years of the workshop, the focus was mainly on DNA extraction, quantitation methods, single-plex assays and gel electrophoresis as well as multiplex platforms to test for CF variants. As the needs of NBS programs have changed and expanded, the workshop now has a strong focus on the real time PCR assays used for primary molecular testing, multiplex genotyping and gene sequencing for second and third tier disorders and liquid handling automation.

The structure of the workshop is designed to provide information on how NBS laboratories use molecular testing as well as to help build a support network across the state program participants, lecturers and CDC’s scientists. The format encourages students to ask questions and provides discussions that are inclusive and engaging. In addition, the participants are strategically paired into laboratory groups of two with CDC staff scientists to ensure individualized attention, in depth discussions, problem solving and relationship building. The student pairs are based on similar assays and workflow in home laboratories and molecular competency levels so they can grow together. The NBS Molecular Training Workshop has been in operation for more than 10 years and has a cohort of 140 participants from 35 NBS programs resulting in increased knowledge and skills in molecular technologies, lasting friendships, collaborations, and endless networking opportunities.

**Presenter:** Laura Hancock, Centers for Disease Control and Prevention, lfn2@cdc.gov
Development of QC and PT Dried Blood Spot-based Quality Assurance Materials for Second-tier LC-MS/MS Methods
M. Kilgore, T. Lim, S. Isenberg, C. Cuthbert and K. Petritis, Centers for Disease Control and Prevention, Atlanta, GA

Introduction: The Centers for Disease Control and Prevention’s Newborn Screening and Molecular Biology Branch (NSMBB) is tasked with providing quality materials to state newborn screening laboratories to assess the performance of newborn screening tests. As more diseases get added to the recommended uniform screening panel and methods change, new method development materials are needed. NSMBB has developed and is currently evaluating dried blood spot-based quality materials enriched with second-tier screening analytes for the following disorders: guanidinoacetic acid methyltransferase deficiency (GAMT), adrenoleukodystrophy (ALD), propionic acidemia (PA), methylmalonic acidemia (MMA), homocystinuria (HCU), and maple syrup urine disease (MSUD).

Methods: Materials were made by enriching blood with analytes of interest and then spotting on filter paper to dry. The second-tier developmental proficiency testing (D-PT) materials consist of the following: GAMT low in creatine (CRE) and enriched with guanidinoacetic acid (GUAC); ALD enriched with lysophosphatidylcholine (LPC) 24:0 and LPC 26:0; PA enriched with methylcitric acid (MCA); MMA enriched with MCA and methylmalonic acid; HCU enriched with homocystine (HCY); and MSUD enriched with alloisoleucine (alle), isoleucine (Ile), leucine (Leu), and valine (Val). To complement the second-tier D-PT materials, second-tier developmental quality control (D-QC) materials are also under development with low, medium, and high enrichments for CRE, GUAC, CRN, LPC 20:0, LPC 22:0, LPC 24:0, LPC 26:0, methylmalonic acid, malonic acid, EMA, MCA, alle, Ile, Leu, Val, and HCY.

Materials were evaluated for homogeneity and characterized using NSMBB laboratory developed assays. On completing internal evaluation, materials were shipped to additional laboratories for interlaboratory evaluation of material performance.

Results: Internal evaluation of second-tier D-PTs and D-QCs gave good homogeneity with acceptable enrichment recoveries. All analytes enriched in D-PT specimens were demonstrated internally to emulate concentrations found in the diseases of interest, with the following characterized values for the D-PTs: GAMT CRE (168 µM) and GUAC (6.47 µM); ALD LPC 24:0 (1.09 µM) and LPC 26:0 (0.923 µM); PA MCA (21.9 µM); MMA MCA (9.30 µM) and methylmalonic acid (142 µM); HCU HCY (26.5 µM); and MSUD alle (24.8 µM), Ile (192 µM), Leu (279 µM), and Val (309 µM). Likewise, the D-QC materials were shown to have concentrations of analytes that span expected healthy and diseased values.

Conclusions: Our internal evaluation concluded that the D-PT and D-QC materials met preliminary requirements. These materials are currently being evaluated by some US public health laboratories with different second-tier screening methodologies to ensure the materials are fit for purpose on multiple testing platforms.

Presenter: Timothy Lim, Centers for Disease Control and Prevention, tlim@cdc.gov
Review of C14.1 Cut-offs for Newborn Screening-targeted Very-long-chain Acyl-CoA Dehydrogenase Deficiency (VLCADD)
A. Brar¹, P. Chakraborty²; ¹Children’s Hospital of Eastern Ontario, Ottawa, ON, Canada, ²Newborn Screening Ontario, Ottawa, ON, Canada

**Background/objective:** Very-long-chain acyl-CoA dehydrogenase deficiency (VLCADD) is a rare fatty acid oxidation disorder marked by a deficiency in a metabolic enzyme, very-long-chain acyl-CoA dehydrogenase. VLCADD is an inherited autosomal recessive disorder leading to life-threatening symptoms during untreated prolonged fasting periods. In Ontario, Canada, VLCADD has been a primary target of newborn screening since 2006. At Newborn Screening Ontario (NSO), the C14.1 acylcarnitine ratio is primarily used to preliminarily diagnose newborns with VLCADD within 3-4 days of birth. The C14.1 cut-off applied is ≥0.65uM for infants ≤7 days of age, and ≥0.40uM for infants >7 days of age. The positive predictive value for this disorder has been relatively low (~8% for the primary target only; ~13% when variant cases of VLCADD are included). The objective of this study is to further analyze the effectiveness of the C14.1 cutoff and to evaluate potential modifications to improve VLCADD screening at NSO.

**Methodology:** Review of NSO Diagnostic Evaluation Report Form (DERFs) of patients referred from 2006 to 2020. Specifically, the effects of raising the cutoff for infants ≤7 days to 0.75uM were analyzed. Additionally, retrospective chart review of neonates diagnosed with VLCADD in Ontario.

**Results:** 165 (~50%) of the 329 babies referred had a C14:1 screening of 0.75uM. Although three infants with variant VLCADD had screening levels between 0.65 and 0.75uM, they did not experience symptoms or require intervention, and some were lost to follow up. Of note, there was a small population of neonates who experienced early decomposition prior to the return of screening results, increasing their risk for mortality or morbidity.

**Conclusion/implications:** In 2021, the C14.1 cutoff at NSO was changed from 0.65 to 0.75uM. It is hypothesized that the modification will reduce the referral rate by half. Completion of the retrospective chart review will provide a qualitative description of early neonatal mortality and morbidity cases. A better understanding of this population will lead to refinements of newborn screening and may enhance management of VLCADD, allowing for the prevention of decompensation prior to screening results.

**Presenter:** Monica Lamoureux, Newborn Screening Ontario, molamoureux@cheo.on.ca

Francis Lee, C. Greene, A. McCabe, A. Moseley, C. Cuthbert and S. Cordovado, Centers for Disease Control and Prevention, Atlanta, GA

All US state programs have implemented newborn screening for Severe Combined Immunodeficiency (SCID) since 2018. As of 2022, 99 US and international labs have enrolled in the CDC proficiency testing (PT) program for SCID. Newborn screening labs use molecular assays to detect abnormally low level of T-cell receptor excision circles (TREC) in blood as a marker for SCID. Each test run requires assurance that the assay performed according to specification. This is shown by quality control (QC) materials that contain specific levels of TREC yielding results that fall within pre-determined ranges. Some labs use QC materials based on custom-made plasmids or synthetic double-stranded gene fragments containing the TREC signal joint sequence. However, supercoiled plasmid amplifies less efficiently than natural TREC, and the purity of synthetic gene fragments is difficult to control or trace. Thus, CDC has created cell-based quality assurance (QA) materials to more closely simulate newborn samples. Traditionally, CDC has prepared TREC-containing dried blood spots (non-SCID controls) from fresh cord blood (FCB) units that have been rejected for transplantation purposes by cord blood banks. One major challenge for large-scale production is obtaining enough FCB within a short period of time. Another issue is acquiring FCB units with the targeted TREC levels suited for QA needs. This requires screening many units and using only the few that are fit for purpose. Such an approach is expensive, inefficient, and wasteful.

As CDC prepares to launch a TREC QC program in addition to the currently offered PT program, we needed an ample and sustainable source of cord blood material to create enough QA DBS. To that end, CDC has developed a new production procedure that uses cryopreserved cord blood nucleated cells (CCBNC) available from a cord blood bank. The process involved an initial acquisition of 2.8e10 cells of CCBNC that can be stored in liquid nitrogen for years. A small 2-fold dilution series of pooled CCBNC suspended in A+ human serum was added to leukocyte-depleted O+ packed red blood cells to prepare dried blood spots (DBS), which were then tested by the TREC assay to measure the amount of TREC as determined by real-time PCR quantification cycle (Cq). Results were plotted in a regression curve to establish the number of cells needed from the CCBNC pool to produce DBS with the target Cq for TREC. This information has allowed us to create three large production lots of DBS, each with different TREC levels from near the population median to low quantities of TREC seen in patients that are immunodeficient. These lots were tested for homogeneity and were determined to be fit-for-purpose based on acceptable target Cq ranges. The new procedure was reproducible, efficient, and affordable, allowing sustainable production of dried blood spots that would enable newborn screening laboratories to ensure accurate detection of TREC.

Presenter: Francis Lee, Centers for Disease Control and Prevention, icr0@cdc.gov
Development of Dried Blood Spot (DBS) Proficiency Testing Materials for Guanidinoacetate Methyltransferase Deficiency and Lysosomal Storage Disorders
T. Lim, M. Kilgore, S. Isenberg and K. Petritis, Centers for Disease Control & Prevention, Atlanta, GA

Background: The Centers for Disease Control and Prevention’s Newborn Screening and Molecular Biology Branch (NSMBB) creates proficiency testing programs and develops quality control materials for recent and anticipated conditions on the recommended uniform screening panel (RUSP). Mucopolysaccharidosis type II (MPS II) was recently voted to be included in the RUSP, while a vote to recommend the addition of Guanidinoacetate Methyltransferase (GAMT) Deficiency to the RUSP is scheduled to take place in May 2022. While NSMBB has provided quality control materials for these disorders since at least 2016, proficiency testing programs for these conditions are not currently available. This presentation will describe efforts related to the preparation of GAMT PT materials by adding Creatine (CRE) and Guanidinoacetic acid (GUAC) and the expansion of the Lysosomal Storage Disorders (LSD) PT program to include not only MPS II but also Gaucher, Fabry and Niemann-Pick A/B disorders that are screened for by several US public health laboratories. CDC is already offering PT materials for Pompe, MPS I and Krabbe LSD disorders.

Methods: Developmental materials for GAMT were made by enriching blood with creatine (CRE) for specimens within normal limits and guanidinoacetic acid (GUAC) for specimens outside of normal limits. Materials were evaluated for homogeneity and characterized internally using flow injection analysis tandem mass spectrometry (FIA-MS/MS). The materials were tested by four newborn screening laboratories. Based on the feasibility study results, target enrichments for CRE, GUAC and ratios of GUAC to CRE for GAMT deficiency DBS were designed for production of GAMT PT pilot materials. Developmental materials for LSD were made by using doubly leukodepleted blood which is deficient in all LSD enzymes. The blood was subsequently aliquoted in 7 parts and each pool was enriched with one recombinant enzyme for the LSD disorders mentioned above at concentrations about 6 times above normal physiological levels. Deficient specimens for each LSD disorder were made by selectively mixing 6 of the 7 blood units together, creating an enzymatic deficiency for the enzyme excluded from the mixture. Developmental materials derived from these blood products were sent for evaluation to selected US newborn screening laboratories.

Results: Internal evaluation of developmental DBS demonstrated good homogeneity with acceptable enrichment recoveries. DBS materials within and outside the normal limits for GAMT specimens were characterized with measurements of CRE (362 µM), GUAC (2.11 µM), ratio of GUACx1,000/CRE (5.8) and CRE (168 µM), GUAC (6.47 µM), ratio of GUACx1,000/CRE (38.5), respectively. Preliminary feasibility study results from 4 external laboratories, demonstrated that the materials were fit for purpose. The evaluation of the LSD materials is in progress.

Presenter: Timothy Lim, Centers for Disease Control & Prevention, tlim@cdc.gov
Development of a Nine-level Dried Blood Spot-based Linearity Material Panel for 43 Newborn Screening Biomarkers
T. Lim, E. Lobo, M. Kilgore, A. Pickens, S. Isenberg, and K. Petritis, Centers for Disease Control and Prevention, Atlanta, GA

Background: The Centers for Disease Control and Prevention’s Newborn Screening and Molecular Biology Branch (NSMBB) assists newborn screening laboratory operations by providing dried blood spot quality assurance materials. During method validation or verification, laboratories need to show linear correlation for all analytes at a dynamic range which should span low healthy percentiles to the highest reported disease percentiles. To accommodate that need, NSMBB has developed a series of 9 level linearity dried blood spot (DBS) based materials that cover a wide concentration range. These materials can help participating laboratories to define their reporting range for the different biomarkers tested.

Methods: A total of 43 analytes in newborn screening (10 amino acids, succinylacetone, guanidinoacetic acid, creatine, creatinine, carnitine and 22 acylcarnitines, 4 lysophosphatidylcholines, and 2 nucleosides) were dissolved in pure solvents in high concentrations to minimize the dilution of hematocrit of the blood. Two blood pools were prepared from packed red blood cells and adjusted with 55% hematocrit with lipid depleted plasma. The high pool was enriched with all 43 analytes at the highest target concentration and the low pool was not enriched. Levels 1-9 of the linearity material were made by mixing contents of the low and high blood pools at ratios of 0, 0.0125, 0.025, 0.05, 0.125, 0.25, 0.5, 0.75 and 1 high/low (v/v). 100µL aliquots of each pool were spotted onto filter paper and dried. Materials were evaluated for homogeneity and characterized using derivatized and non-derivatized FIA-MS/MS assays.

Results: Recoveries for all 43 analytes were within expected target ranges of historical values for DBS. All 43 analytes have shown linearity with R2 values of 0.994 or higher for both derivatized and non-derivatized characterization with concentrations ranging from 0.1 to 2,000 µM.

Conclusions: The development of a nine-level DBS linearity panel, prepared by mixing high and low pools to specified ratios, was successful. The materials can be used to assure that newborn screening assays are linear over the desired reporting range. These materials are currently available by request for Newborn Screening Quality Assurance Program (NSQAP) participants.

Presenter: Timothy Lim, Centers for Disease Control & Prevention, tlim@cdc.gov
Investigating Strategies for Overcoming the Challenges of Low and Non-homogeneous Biotinidase Activity in Dried Blood Spot Based QA Specimens

E. McCown and K. Petritis, Centers for Disease Control and Prevention, Atlanta, GA

Biotinidase (BIO) deficiency is an inherited disorder where the patient’s body is unable to recycle their supply of biotin. In the newborn screening (NBS) community, the BIO enzyme has gained a well-deserved reputation for being notoriously labile. It is not uncommon for NBS laboratories to experience increases in false-positives for BIO deficiency during summer months and, more sporadically, when transitioning to new lots of collection cards. As a provider of NBS quality assurance (QA) specimens, the fickle nature of BIO also causes problems for the Newborn Screening Quality Assurance Program (NSQAP) at the CDC. One of the greatest challenges has been achieving homogeneity of BIO enzyme activity in a single lot of DBS QA materials. We have repeatedly seen wide variations in activity from card-to-card—and even from spot-to-spot on a single card—but without any apparent, repeatable pattern. The problem is so significant that it has prevented CDC from being able to provide suitable quality control (QC) materials for BIO to NSQAP participants; currently, we distribute only proficiency testing (PT) materials for BIO.

In this study we investigated several parameters that may affect BIO activity after spotting to filter paper and devised strategies that could potentially overcome those issues. Parameters investigated in this study were drying time, humidity during drying, filter paper lot, pretreatment of filter paper before spotting and use of recombinant BIO enzyme. Preliminary results indicate that overall BIO activity as well as homogeneity can be improved without the need to use recombinant enzymes. The contribution of each parameter on the BIO stability after spotting will be discussed. Having this information will allow us to better serve the NBS community by providing reliable QC materials for BIO for the first time.

Presenter: Elizabeth McCown, Centers for Disease Control and Prevention, erm5@cdc.gov
Effect of Methanol Quenching on Newborn Screening for Succinylacetone Analysis in Dried Blood Spots
D. Peppers, A. Pickens, C. Cuthbert and K. Petritis, Centers for Disease Control and Prevention, Atlanta, GA

Newborn screening (NBS) of dried blood spots (DBS) using flow injection analysis tandem mass spectrometry (FIA-MS/MS) has been successful in measuring biomarkers associated with inborn errors of metabolism. Succinylacetone (SUAC) is a biomarker associated with Tyrosinemia Type 1 (TYR 1). Current methods allow for extraction and analysis of SUAC by incubation with hydrazine to form a hydrazone derivative, which enhances ionization and detection of SUAC by FIA-MS/MS. Some laboratory developed tests (LDTs) have a step post hydrazine derivatization, where methanol is added to DBS extracts for the purpose of quenching any residual unreacted hydrazine. While the intention of methanol quenching was to prevent fluctuating SUAC-hydrazone concentrations over time, some methods use methanol-based extraction solutions that contain hydrazine, which put into doubt the requirement of a methanol quenching step. The purpose of this study was to test whether removal of methanol quenching impacted SUAC-hydrazone concentrations. The samples used in this study were Newborn Screening Quality Assurance Program (NSQAP) quality control (QC) materials. 1/8” (3.1 mm) DBS spots were punched into 96 well plates. We processed the samples using an in-house developed first-tier screening method that uses 80:20 acetonitrile/water, formic acid, hydrazine, and isotopically labeled internal standards and utilizes a methanol quenching step. For comparison, we prepared an additional four 96 well plates containing our QC specimens and used our first-tier screening method without the methanol quenching step. Four plates of each method were prepared, totaling approximately 380 samples per method. The four plates per method were analyzed sequentially by FIA-MS/MS on separate days. We report no linear relationship between SUAC-hydrazone concentration and the time of injection when removing the methanol quenching step. Between both groups, SUAC concentrations were similar across the 384 samples (768 samples total). This indicates that methanol quenching may not be needed as a post extraction step, as it does not impact SUAC concentrations. The omission of the methanol quenching step would simplify methods and increase throughput.

The findings and conclusions in this study are those of the authors and do not necessarily represent the official position of the U.S. Department of Health and Human Services, or the U.S. Centers for Disease Control and Prevention (Division of Laboratory Sciences). Use of trade names and commercial sources is for identification only and does not constitute endorsement by the U.S. Department of Health and Human Services, or the U.S. Centers for Disease Control and Prevention (Division of Laboratory Sciences).

Presenter: Daquille Peppers, Centers for Disease Control and Prevention, syy7@cdc.gov
P-21

Health Economic Evaluation and pan-Canadian Collaboration for Spinal Muscular Atrophy (SMA) Newborn Screening
A. Wyatt¹, P. Chakraborty²; ¹Children Hospital of Eastern Ontario, Ottawa, ON, Canada, ²Newborn Screening Ontario, Ottawa, ON, Canada

Background: Societal considerations are important in decision making about additions to NBS panels, including impacts on health systems and services. Health economic considerations are often particularly important to health system decision makers. Canadian-specific and high-quality health economic analyses are often not available. Further, there is a lack of infrastructure and support to share NBS information, policies and practices across provinces and territories. Each sub-national jurisdiction is responsible for their own newborn screening programs as health is not in Federal jurisdiction. We have therefore initiated a course of work that will generate Canadian health economic information to support NBS policy decision making in Canada and mechanisms for knowledge sharing.

Methods: The project will identify and leverage existing Canadian rare disease research networks to generate the Canadian health economic information needed to support the addition of SMA to screening panels across all provinces and territories. Further, we are developing an infrastructure to support collaboration between each Canadian sub-national jurisdiction on NBS policies and procedures.

The specific steps are to:
• 1. Perform an updated literature review for new publications relevant to cost effectiveness and societal impacts of NBS for SMA
• 2. Define key outcomes and data elements to collect longitudinally in Canada for both screen-ascertained and clinically diagnosed patients with SMA.
• 3. Conduct a costing analysis of screening strategies to permit calculation of the budgetary impact for routine screening.
• 4. Develop a model and evaluate the cost-effectiveness of universal NBS for SMA in Canadian jurisdictions, including early treatment with Zolgensma for screen-identified children with SMA.
• 5. Create a means for communication and collaboration across Canada vis a vis NBS policy, program implementation, and evaluation.

Results: We have identified existing rare disease research networks with whom to pursue our research objectives: INFORM RARE and the Canadian Neuromuscular Disease Registry (CNDR) are working to address patient/caregiver- and clinician- reported SMA outcomes through longitudinal registry data. We have integrated our objectives to create a shared research agenda and have initiated model development, definition of data elements, and means of collection needed for the economic analysis for NBS for SMA.

Implications: Canadian-specific health economic evaluation will facilitate NBS health policy decisions in provinces not currently screening for SMA, and will assist provinces currently screening to further improve the impact and efficiency of their programs. This will be especially important in an evolving treatment landscape.

Presenter: Pranesh Chakraborty, Newborn Screening Ontario, pchakraborty@cheo.on.ca
Pivoting to Telegenetics for Sickle Cell Trait Newborn Screening Counseling and Education
L. Shook, D. Haygood, C. Mosley and C. Quinn, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Introduction: Telegenetics is a delivery model of counseling and education using web-based video. Studies have demonstrated benefits such as improved efficiency of time and increased access to care for patients with cancer and other genetic disorders. However to date, telegenetics and sickle cell trait (SCT) has not been extensively studied. All newborns are tested for hemoglobin disorders at birth through newborn screening (NBS). In Ohio, newborns with suspected sickle cell trait are referred to a regional sickle cell program for confirmatory testing, counseling and education. As an immediate response to COVID-19 in spring 2020, the NBS team began piloting telegenetics for SCT. Previously, families were contacted by phone to coordinate confirmatory testing, education and counseling.

Methods
Parents of newborns identified by the Ohio Department of Health with suspected SCT in the southwestern region of Ohio were eligible to participate in the pilot. Caregivers could choose their preference of phone or online video counseling. There was no eligibility exclusion criteria. Because this was an immediate clinical response to COVID-19, this activity did not require IRB approval. Caregivers agreed to a scheduled appointment time and a unique meeting link to Zoom (HIPAA-protected edition) was sent to their email. During the session, a polling feature administered five pre-test knowledge questions to assess the parents’ baseline knowledge of SCT. Then the NBS coordinator gave a tailored Powerpoint presentation based on the newborn’s hemoglobinopathy test result. At the end of the session, a demographic and satisfaction survey was launched with the polling feature.

Results: Since March 2020, 75 families participated in telegenetics counseling and education. Nearly 76% of the participants were African-American, 46% were ages 25-34, with the highest level of education being some college with no degree (29%). Participants demonstrated statistically significant increases in three key knowledge areas of SCT education, including the misconceptions of “only African-American newborns are screened for sickle cell trait,” “SCT is a mild form of disease,” and the fundamental concept of “If both parents have SCT, what are the chances, with each pregnancy, of having a child with sickle cell disease.” Participants reported satisfaction with the virtual format (82%), presentation visuals helpful (82%), and technology easy to use (92%). The majority of participants used a smart phone (68%) to connect to Zoom.

Discussion: Preliminary results of this pilot show promise for utilizing telegenetics for SCT counseling. This format has been helpful for families to receive interactive education and counseling and integrates adult learning principles using visual aids (i.e. Powerpoint), and provides the ability to collect real-time knowledge, demographic and satisfaction data.

Presenter: Lisa Shook, Cincinnati Children’s Hospital Medical Center, lisa.shook@cchmc.org
Machine learning (ML) is an evolving topic within the data science and bioinformatics communities, with new research topics and discoveries being published rapidly within all disciplines of science and research. ML pulls from multiple disciplines including statistics, coding, and data science in a unique way that shows great promise for use in disease predictability, trends, outcome research, and forecasting. Recently, ML has been applied to complex genomic and sequencing data to identify novel insights and genomic regions of focus, as well as using biochemical analytes in disease prediction. A key strength of ML is the ability to pull from multiple sources and find insight into relationships which would be difficult to discover and consider without such tools.

Due to these advancements, there is an opportunity to utilize ML on behalf of the Newborn Screening (NBS) community to incorporate patient-level clinical, biochemical, and genomic data. A primary goal of the NBS program is the early identification of disease state in newborns and communication of such states to patients and clinicians to ensure proper care of the newborn. Current challenges to effective use of ML for NBS include data harmonization and standardization across states which each use their own measures of data and outcome reporting. The CDC Newborn Screening and Molecular Biology Branch is developing a platform, ED3N, which aims to bring transparency to the NBS disease identification process. While currently in the pilot stage, the goal of ED3N is a national platform through which screeners and clinicians are given clear, transparent, and understandable risk assessments, and through which standardization and harmonization across all state NBS programs can be made possible.

Incorporation of ML into NBS and the ED3N platform is a key focus area and long-term goal of ED3N. Due to the growing momentum of ML, incorporating evolving ML techniques may outperform current prediction accuracy and validity found using other methods. ML can be utilized to identify additional patterns or gene regions of interest which could add additional information and perspective within the NBS disease prediction process.

This presentation will highlight current research on ML, its role and promise in predicting disease state, as well as outlining its potential for NBS ED3N platform and NBS disease prediction.

**Presenter:** Amy Gaviglio, Connetics Consulting/CDC/APHL, amy.gaviglio@outlook.com
P-24

Guiding the Worldwide Newborn Screening Community: An Update on CLSI Products for Newborn Screening Programs
A. Gaviglio¹, L. Moon²; ¹Connetics Consulting/CDC/APHL, Minneapolis, MN, ²CLSI, Malvern, PA

The Clinical and Laboratory Standards Institute (CLSI) provides standards and guidelines for professionals, developed through a unique consensus process that incorporates the values of inclusiveness, excellence, responsiveness, integrity, and teamwork. The collaborative approach includes balanced representation from members of industry, government, and health care professions throughout the world.

CLSI has facilitated the development of Newborn Screening (NBS) standards and guidelines since 1988 when the first NBS standard, Blood Collection on Filter Paper for Newborn Screening Programs, was published. Since 1988, CLSI’s NBS standards have grown to eight, with more currently in development. In the last two years alone, four NBS standards have been revised or developed. To date, five more are in the process of being revised and developed. These standards cover a wide variety of NBS topics and discuss pre-analytical, analytical, and post-analytical phases of the NBS system.

This presentation will provide an overview of the topics covered by these standards, including any changes to guidance, and a discussion of available ancillary products. Specifically, this presentation will provide an overview of the following guideline topics:

- Blood Collection on Filter Paper
- NBS Follow-Up
- NBS for Preterm, Low Birth Weight, and Sick Newborns
- Overarching Program Implementation and Evaluation
- NBS for the following diseases:
  - Cystic Fibrosis
  - SCID and Other Related Immunodeficiencies
  - Hemoglobinopathies
  - X-linked Adrenoleukodystrophy
  - Congenital Hypothyroidism
  - Congenital Adrenal Hyperplasia
  - Galactosemia
  - Spinal Muscular Atrophy
  - Lysosomal Disorders

Ongoing and future initiatives from CLSI will also be discussed, inclusive of terminology harmonization efforts, cost analysis in NBS, and additional ancillary products for CLSI readership.

As the complexity of NBS continues to grow, it is more and more important for programs around the world to utilize CLSI standards and guidelines.

Presenter: Amy Gaviglio, Connetics Consulting/CDC/APHL, amy.gaviglio@outlook.com

APHL 2022 Newborn Screening Symposium – October 16-20, 2022 – Tacoma, WA
Proximal urea cycle disorders (PUCD) are a group of three rare genetic disorders which can present with a life-threatening hyperammonemic crisis early in infancy leading to an ongoing evaluation by NBS programs to consider effective screening strategies. These disorders include N-acetylglutamate synthase (NAGS), carbamoyl phosphate synthase (CPSI), and ornithine transcarbamylase (OTC). We conducted a pilot screening project to evaluate the possibility of detecting PUCDs using the CLIR post-analytical tools in the Georgia newborn screening program. We used existing data from the amino acid results obtained on the underivatized Neobase2 kit from Perkin Elmer. We began screening samples in October 2019, paused in March 2020 due to the COVID-19 pandemic, resumed in December 2020, and ended in September 2021. We evaluated 138,560 NBS samples (between 125,000 and 130,000 children). Screens were reported as abnormal if the CLIR score fell into a higher risk category. Due to the large number of false positives and the discovery that lysine is contained in the glutamine transition using the underivatized Neobase2 kit, we made adjustments to the single condition tool and the follow up protocols, and also created dual scatter plot tool. We reported had 562 samples flag as positive from the CLIR single condition tool. The dual scatter plot further reduced this to 450 needing follow up in some form. Each case was resolved as follows: 171 by confirmatory testing, 262 by subsequent normal NBS, 70 by prior normal NBS, 42 closed based on a combination of clinical assessment and age at collection of one month of age or older making a PUCD unlikely, and 17 were lost to follow-up. We did not diagnose any cases of PUCD nor are we aware of any cases diagnosed clinically during the screening period nor during the pause in screening. We do not recommend PUCDs be added to NBS panels at this time given our experience. It is possible that a different lab methodology which separates glutamine from other amino acids may produce better results. We also experienced challenges with primary care providers understanding of these disorders and test results. The urgent nature of the disorders and need for timely follow up was not always well received, and providers struggled with appropriate reference ranges for ammonia levels in infants, and previously known pre-analytical issues with ammonia measurement.

**Presenter:** Angela Wittenauer, Emory University, alwitte@emory.edu
Expecting Health’s Newborn Screening Genetic Counseling Internship: Creating Opportunities for Education and Collaboration around Newborn Screening for Genetic Counseling Students
M. Raia¹, N. Bonhomme¹, Brianne Miller²; ¹Expecting Health at Genetic Alliance, Damascus, MD ²Children's National Hospital, Washington, DC

In 2022, Expecting Health launched its Newborn Screening Genetic Counseling Internship program for current genetic counseling students. This program comes as the need for genetic counselors in the newborn screening system grows and aims to increase knowledge of the NBS system in this population prior to their entering the workforce.

A call for applications was shared with genetic counseling program directors across the United States. Program directors were directed to share this opportunity with their students and interested students were subsequently invited to apply through an online application.

During the 4-week internship, which will occur during June 2022, interns will spend 4-6 hours per week learning from experts in NBS and public health genetics and their peers about key topics in NBS. Internship activities will consist of synchronous learning sessions (lecture and group discussion), one-on-one mentoring and support with Expecting Health staff, and self-guided research projects about newborn screening in their state. The program will assess participants' awareness and knowledge of newborn screening and the beginning and end of the 4 week program.

In this presentation, we will share available data as well as our experiences with recruiting and educating future genetic counselors about NBS and public health genetics and the roles they can play in improving the system.

**Presenter:** Marianna Raia, Expecting Health, mraia@expectinghealth.org
Reaching the Unreached: A Flexible Education Model for Expecting Mothers
M. Raia¹, N. Bonhomme¹, B. Miller², J. Werker³; ¹Expecting Health at Genetic Alliance, Damascus, MD, ²Children's National Hospital, Washington, DC, ³Indiana Community Health Clinic, Topeka, IN

Successful education and engagement of families requires a multifaceted approach including involvement at the local, state and national levels. Despite a significant body of literature, as well as guidance from professional societies such as ACOG, stating that education for newborn screening ideally occurs during pregnancy, this is not common practice among prenatal providers today (*1-7). Barriers to prenatal education implementation have historically included provider lack of comfort and confidence to provide information to patients and/or clients around newborn screening. Additionally, prenatal care providers as well as families are inundated with information during pregnancy making time for newborn screening education challenging to implement during this critical time period.

The Newborn Screening Family Education Program is dedicated to developing opportunities for all families to learn about newborn screening as well as training and educational resources that build confidence for families to become leaders in the newborn screening system. In an effort to increase awareness and knowledge of newborn screening in pregnant people, the program established a model for delivering newborn screening education in prenatal settings. The program piloted the model in three unique communities supporting families from medically underserved areas including 1) a Latino group in Houston, TX 2) Plain Community in Indiana 3) Tribal Community in Oklahoma.

The prenatal newborn screening education initiative includes pre and post test assessments of awareness and knowledge followed by education through an online or paper copy of the Navigate Newborn Screening flipbook. Through comparison of pre and post test responses, this initiative has successfully shown increases in participants awareness and knowledge of newborn screening. Since piloting, the program has observed increased interest in implementing scalable models of prenatal education initiatives across multiple state NBS laboratories, clinics and prenatal groups.

During this presentation, we will review the strategies and tools used to develop this pilot program, we will share available data on the outcomes of success and we will discuss considerations for how state newborn screening programs can partner to implement prenatal education initiatives.

**References available upon request.

**Presenter:** Marianna Raia, Expecting Health, mraia@expectinghealth.org
Creating a Model for Collaborating around New Conditions in Newborn Screening: A Congenital Cytomegalovirus and Newborn Screening Work Group

B. Miller¹, N. Bonhomme²; ¹Children's National Hospital, Washington, DC, ²Expecting Health at Genetic Alliance, Washington, DC

Congenital cytomegalovirus (cCMV) has been nominated for review to be added to the Recommended Uniform Screening Panel (RUSP) and added to at least one state’s newborn screening (NBS) panel. However, many questions remain about the logistics of adding cCMV to NBS programs across the United States.

In March 2022, Expecting Health began hosting quarterly meetings of the CONECT work group, a group specifically tasked with collaborating around the potential integration of cCMV into routine NBS, to ask and answer those hard questions. The group is comprised of members of varying expertise in newborn screening and CMV prevention including parents, healthcare providers, researchers, state program representatives, industry representatives, and advocacy and education organization representatives.

In this presentation, we will highlight the discussion and work of the group to date, continuing the conversation with the larger community. Specifically, we will outline the enablers and barriers to screening for cCMV through NBS, highlight areas for continued work and collaboration, and share what the work group sees as the next steps towards success. For example, the group has identified several existing and needed supports for successful cCMV screening. These include funding and opportunities for research, similarities and differences between cCMV and other NBS conditions, and testing and follow-up methods.

This work group serves as a model for collaboration and problem solving around the integration of new conditions into routine newborn screening prior to their addition to state panels and the RUSP.

**Presenter:** Natasha Bonhomme, Expecting Health at Genetic Alliance, nbonhomme@expectinghealth.org
The Newborn Screening Information Center: A National Newborn Screening Education Website
K. Sprunck¹, B. Miller², A. Harris³, A. Keehn³, N. Bonhomme⁴; ¹National Institute for Children's Health Quality, Boston, MA, ²Children's National Hospital, Washington, DC, ³Health Resources and Services Administration, Washington, DC, ⁴Expecting Health at Genetic Alliance, Washington, DC

In 2018, the Health Resources and Services Administration (HRSA), Department of Health and Human Services contracted the National Institute for Children’s Health Quality to lead the creation of the Newborn Screening Information Clearinghouse, The Newborn Screening Information Center (NBSIC); Genetic Alliance was subcontracted for their content expertise. The NBSIC provides clear and up-to-date information, materials, and resources about newborn screening (NBS) in the United States. These resources help increase awareness, knowledge, and understanding of NBS processes, conditions, and why it is important. The website was developed and is maintained by the Health Resources & Services Administration's Maternal and Child Health Bureau (MCHB), per 42 USC § 300b-11. All content on the NBSIC is reviewed by experts and in updated quarterly.

The purposes of this site are to:
• Define NBS, describe the NBS process, and explain how that process relates to follow up, diagnosis, and treatment
• Identify the conditions included as part of NBS
• Provide state specific information
• List the types of NBS results and describe what happens after screening for babies with each type of result
• Connect parents, parents-to-be, and health care providers with state-specific NBS resources
• Help readers learn about updates in NBS

Presenter: Natasha Bonhomme, Expecting Health at Genetic Alliance, nbonhomme@expectinghealth.org
What has been the Impact of a 17-year Comprehensive NBS Program in Mexico?
H. Cruz-Camino, C. Cantu-Reyna, C. Araiza-Lozano, Diana Laura Vazquez-Cantu and R. Gomez-Gutierrez, Genomi-k, Monterrey, NL, Mexico

Introduction: The inborn errors of metabolism (IEM) are a group of more than 1,000 rare inherited disorders. Newborn screening (NBS) is fundamental for the early diagnosis of IEM and other disorders. Currently, there is still a challenge upon coverage, implementation uniformity and evaluation, causing an uncertain panorama of IEM prevalence. Therefore, we present the results of a private NBS program recorded to date with the broadest panel available in Mexico (50+ IEM and other disorders), on account of the use of a specialized software developed in-house for patient information management.

Objective: To report the results of Genomi-k’s NBS program in Mexico providing a surrogate prevalence of screened disorders.

Materials and Methods: We retrospectively analyzed 321,900 NBS dissociated results emitted as of October 2004 to June 2021, including only reports with defined demographics. All DBS were processed by PerkinElmer Genomics and abnormal results were followed by diagnostic tests.

Results: In the 17 years, our program presented an overall disorders prevalence of 4.5:1,000 screened NB. The most frequent diagnoses were: G6PD (72.32%), CH (11.38%), and CAH (1.79%). On the other hand, the group of six LSD showed the highest prevalence in comparison to other groups. More than 50% of newborns with an abnormal finding in their initial screening result comprehended only amino acid and acylcarnitine profiles, G6PD, as well as Hemoglobinopathies. In this experience, our NBS had a high specificity (99.97%), sensitivity (near 100%), and positive predictive value (76%). Regarding the newborns that obtained a first screening abnormal result, 7.6% were lost to follow-up.

Discussion and Conclusions: Our reported prevalence may be higher than those reported in the literature, mainly due to a broader screening panel and population type –4.5 vs 1.25 per 1,000 NB–. The report of extensive epidemiological studies with RUSP-based programs will show a clearer picture of the distribution and benefits of IEM screening. In addition, strategies and efforts to reduce the NB lost to follow-up are key to attain an early therapeutic approach.

Presenter: Hector Cruz-Camino, Genomi-k, hcruz@genomi-k.com
Analytical Performance Evaluation of the TaqMan SCID/SMA Plus Assay in Newborn Dried Blood Spots (DBS)

J. do Prado Silva\textsuperscript{1}, C. M. Crua da Silva\textsuperscript{2}, D. Alves Gomes Zauli\textsuperscript{1}, A.C. Perssonelli Sera\textsuperscript{2}; \textsuperscript{1}Instituto Hermes Pardini, Vespasiano, Minas Gerais, Brazil, \textsuperscript{2}Diagnósticos Laboratoriais Especializados

\textbf{Introduction:} Severe Combined Immunodeficiency (SCID) is a genetic disease that may compromise the Lymphocytes T, B, and/or Natural Killers cells functions. Low levels of the recombination excision circles of T and B cells (TREC and KREC, respectively) are indicative of SCID or other lymphopenias. In addition, Spinal Muscular Atrophy (SMA) is a neurodegenerative disease caused by the homozygous deletion involving exon 7 of the SMN1 gene in 95\% of cases. This mutation causes the degeneration of motor neurons, resulting in muscular atrophy. Both SCID and SMA affect newborns and can be lethal if not early diagnosed. Therefore, diagnostic methods capable of accurately identifying these genetic diseases is extremely important in the newborn screening.

\textbf{Objectives:} To describe the analytical validation of the TaqMan SCID/SMA Plus Assay (Thermo Fisher, Massachusetts, EUA) for the implementation of a multiplex SCID/SMA real-time diagnoses routine in dried blood spots (DBS).

\textbf{Methods:} DNA isolation was performed with the DNA Extract All Reagents Kit (Thermo Fisher, Massachusetts, EUA) from a 3.2mm disc of newborn DBS. Assay performance was evaluated using commercial quantified positive control to TREC, KREC, and SMN1 targets. The analysis parameters included: (i) Assay standardization; (ii) Determination of assay efficiency; (iii) Analytical sensitivity (Limit of detection); and (iv) Intrassay and interassay precision. All reactions included the endogenous control RNase P.

\textbf{Results:} The assay demonstrated a reaction efficiency of >98\% and the limit of detection was 30 copies per reaction for TREC, KREC, and SMN1 targets with a 95\% confidence interval. The regression equations obtained show good amplification conditions with a positive correlation between the variables, with a coefficient of determination (r\textsuperscript{2}) of 0.99. The experiments performed to evaluate the precision demonstrated optimal repeatability and reproducibility.

\textbf{Conclusion:} An accurate and rapid diagnosis is essential to guide the clinical management of SCID and SMA in newborns. Here, we evaluated and described the TaqMan SCID/SMA Plus Assay performance, a molecular test for detecting SCID and SMA by Quantitative Real-time PCR (qPCR). This assay was a highly efficient due offer of a multiplex detection method, allowing the rapid release of results. The use of this new tool can help the clinicians in the rapid newborn screening, providing agility in clinical conduct. New tests on clinical validation are being carried out to evaluate the assay performance in clinical samples.

\textbf{Presenter:} Joice Silva, Grupo Pardini, joice.silva@grupopardini.com.br
Overnight Shipping Trial for Midwives and Smaller Facilities to Improve Transit Time from Collection to Receipt for the State of Kansas Newborn Screening Program
M.J. Mills¹, B. Adhikari²; ¹Kansas Department of Health and Environment, Topeka, KS, ²Centers for Disease Control and Prevention, Atlanta, GA

Through the Association of Public Health Laboratories (APHL), the State of Kansas received a quality improvement (QI) grant in 2020 to improve transit time between collection at facilities and receipt at the Newborn Screening (NBS) Laboratory. Utilizing the APHL QI grant, we have implemented a courier for Sunday pickups of NBS specimens for the 29 Kansas facilities with the highest submission numbers. In 2021, we wanted to continue our work on improving transit time, and APHL proposed a different option for those smaller facilities and midwives. The State of Kansas offered a 6-month trial to provide overnight shipping labels, free of charge, to 62 smaller facilities and 31 midwives. We monitored and analyzed transit time from collection at those facilities and midwives to receipt at the NBS laboratory during the 6-month trial.

During the trial period, the State of Kansas implemented a daily courier to pick up any laboratory specimen from public health departments. Because of the new daily courier, the overnight shipping trial was not extended to the 62 smaller facilities after the trial ended. Due to the resource limitations and high transit time for midwives, the overnight shipping trial was extended for midwives only. The data presented in this poster demonstrates the transit time for midwives during and after the trial. The data also shows the continued transit time for the 62 smaller facilities after the overnight shipping trial ended.

Presenter: Michelle Mills, Kansas Department of Health and Environment, michelle.j.mills@ks.gov
Review of Effectiveness of the Newborn Screening in Kentucky
H. Stone\textsuperscript{1}, L. Mott\textsuperscript{1}, S. Wei\textsuperscript{2}, A. Smith\textsuperscript{1}, D. O’Quinn\textsuperscript{1}, M. Yu\textsuperscript{1}; \textsuperscript{1}Kentucky Division of Laboratory Services, Frankfort, KY, \textsuperscript{2}University of Kentucky, Lexington, KY

Introduction: Newborn screening identifies infants at high risk for congenital disorders, which allows an early intervention and leads to improved outcomes. In newborn screening laboratory at the Department of Public Health in Kentucky State (KY), 55 primary conditions are screened, increased from 53 since year of 2017. The prevalence of Newborn Disorders identified in KY population has not been described previously.

Methodology: 4 years of Newborn screening data (2017-2020) was reviewed and evaluated to determine the prevalence of Newborn Disorders for the state of Kentucky. The newborn screening data contains both initial newborn screening results and confirmation results from referral.

Results: Overall, of a total of 202,988 newborns screened, the disorder prevalence is \textasciitilde1 in 400 birth. The prevalence of individual disorder distributed in a wide spectrum. Among all disorders we screened, the prevalence of Congenital Hypothyroidism (CH) is the highest, which is 1 in 931 babies.

Conclusions and/or implications: Our results provide the scope of impact of the newborn screening on the public health in the state of KY.

Presenter: Amy Smith and Daniel O’Quinn, Kentucky Division of Laboratory Services, lea.mott@ky.gov
Multiplexed LC-MS/MS Proteomic Pilot Study for Newborn Screening of Wilson Disease and Inborn Errors of Immunity in Washington State
C. Collins¹, A. Meuser¹, S. Sandin², C. Klippel¹, A. Singh², S. Shaunak², J. Hill², T. Shahbal², J. Uchytil², B. Officer², R. Dayuha³, P. Duong³, J. Thompson², S. Hahn³; ¹Key Proteo, Inc., Seattle, WA, Washington State Department of Health, Shoreline, WA, Seattle Children's Research Institute, Seattle, WA

NBS is considered an extremely successful public health program in identifying infants with treatable disorders for early intervention with favorable outcomes. Unfortunately, for many congenital disorders there are no specific metabolic biomarkers nor any analytical methods suitable for population screening even where highly effective treatments are available. In congenital disorders, the causative mutations often result in reduction or absence of their associated proteins. In these cases, direct measurements of these proteins using multiplexed proteomic LC-MS/MS methods from dried blood spots can be diagnostic and utilized in population screening.

Direct measurement of surrogate peptides has been shown to be a sensitive and specific screening method for the multiplex detection of patients with WD and three life-threatening inborn errors of immunity, X-linked agammaglobulinemia (XLA), Wiskott-Aldrich syndrome (WAS), and Adenosine Deaminase deficiency (ADAD) from dried blood spots (DBS). We found that quantification of ATP7B signature peptides effectively identified WD patients in 92.1% of cases and reduced ambiguities in ceruloplasmin and genetic analysis. Similarly, this method was able to identify molecularly confirmed cases of XLA, WAS and ADAD in DBS. Analysis of signature peptides found statistically significant reduction or absence of peptide levels in affected patients compared to control groups. Each of these disorders results in severe negative sequelae if untreated but are treatable if diagnosed early in life.

A first-of-its-kind proteomic-based IVD kit has been manufactured with all necessary reagents to identify these four new conditions in a single-run multiplex assay from DBS. As of January 2022 a pilot study is underway, in conjunction with the WA State public health newborn screening laboratory, to screen 50,000 newborns for the targeted disorders. At time of submission, 5,083 newborn samples had been screened. All were negative for the target conditions. Pilot study results will define the normal ranges of these target peptides in newborns generally and will be sub-divided by gender, ethnicity, and birthweight. This study will validate both the feasibility of newborn screening for these conditions and the use of multiplexed LC-MS/MS proteomic analysis as an effective methodology for population screening.

Presenter: Christopher Collins, Key Proteo, chris.collins@keyproteo.com
Monitoring Birthing Hospitals’ Best Practices through Performance Measurement Scorecard Implementation in Louisiana

N. Huynh¹, C. Harris¹, J. Herwehe², M. Brewer², T. Ibieta²; ¹Louisiana Genetic Disease Program, ²Louisiana Bureau of Family Health

**Background:** Louisiana Early Hearing Detection and Intervention (LA EHDI) program had been successfully sending scorecards of infant hearing testing performance measures to birthing hospitals. This tool helps birthing facilities develop and implement quality hearing screening programs that meet best practice standards and Louisiana legislative mandates. In 2021, Louisiana’s Genetic Diseases programs collaborated with LA EHDI and the breastfeeding program to produce the newborn screening scorecard for each birthing hospital in the state for the first time. This scorecard has performance domains for LA EHDI and Genetic Diseases screening and breastfeeding initiation. This abstract only focuses on the Genetic Disease screening that aligns with the scope of the Association of Public Health Laboratory Newborn Screening Symposium.

**Issues/Challenges:** The Genetic Diseases program aims to eliminate or reduce mortality, morbidity and disabilities by early detection and treatment of the 34 disorders included in the newborn screening panel. To achieve this goal, the program will first need to ensure every newborn receives a valid heel stick newborn screen. Invalid Newborn screening (NBS) tests will delay appropriate follow up treatment of the infants with these disorders. In Louisiana, one of the most common reasons for invalid NBS tests is unsatisfactory specimens. Monitoring NBS and Unsatisfactory rates by each birth hospital will help facilities monitor their best practices and identify opportunities for improvement.

**Approach:** The Genetics Disease program analyzed NBS rates by hospital. 2020 NBS data was matched with Birth data from State Vital Records using LinkPro SAS program version 9.4. This process identified the number of screened infants (numerator) among number of babies born in each birthing hospital (denominator) to generate the NBS rates. The unsatisfactory rates were conducted based on number of infants with unsatisfactory initial screenings among tested infants for each birthing facilities. Besides the NBS and unsatisfactory rates of each facility, the scorecard also included the State’s average score for performance comparison. Fifty-five (55) scorecards were sent to 55 birthing hospitals throughout the state in December of 2021.

**Results/Lesson learned:** Performance scorecards were well received by Birthing hospitals have inquired about how to collect better specimens for newborn heel stick screening to improve unsatisfactory rates. A video on proper collection techniques of NBS specimens was posted on the Genetic Diseases program’s website for reference. One hospital was able to correct the specimen collection process to reduce its unsatisfactory NBS rate by 34 percent points. Continuing to produce score cards yearly can help improve NBS rates and reduce unsatisfactory rates among birthing hospitals.

**Presenter:** Ngoc Huynh, Louisiana Genetic Diseases Program, ngoc.huynh@la.gov
Interoperability in Minnesota: Electronic Laboratory Orders and Results for Newborn Blood Spot Screening
K. Bye, H. Brand, H. Winslow, J. Simonetti, M. McCann, T. Heaney and M. Doerr, Minnesota Department of Health

The Minnesota Department of Health (MDH) newborn screening (NBS) program understands that timely and accurate information is especially vital to the newborn blood spot screening process, as some of the disorders targeted by newborn screening can present within days of birth. Early diagnosis and treatment are imperative, with the best outcomes for baby occurring pre-symptomatically. Due to the many challenges that come from the manual work that accompanies the newborn screening process, the MDH NBS program has worked with a pilot hospital to receive electronic laboratory orders and return electronic results for newborn blood spot screening. The automation through electronic solutions will benefit Minnesota families, birth hospitals, clinics, and newborn screening program staff.

This interoperability project utilized HL7 standard messaging and the MDH information data exchange for electronic newborn screening test ordering and reporting. All patient demographics and specific lab order information were received from the hospital’s electronic health record (EHR) system by the NBS Laboratory Information Management System (LIMS). The NBS LIMS system then sent final screening results to the hospital EHR for consumption and to close the loop on patient data. MDH referenced the standard HL7 version 2.5.1 LOI and LRI Implementation Guides constrained to meet the needs of the MN NBS program. Project activities, challenges, and lessons learned will be included in the poster/presentation.

Benefits of this project include:

- Increased accuracy of patient demographic data as well as NBS results entry into EHR
- Eliminated manual work for hospital staff who handwrite data fields on NBS cards
- Decreased NBS staff time spent manually entering data from NBS card into LIMS and requesting missing information from hospital*
- Decreased hospital staff time with fewer requests for report amendments* and manual entry of NBS results into EHR*

Future plans to expand newborn screening interoperability in MN include:

- Developing and implementing a plan for onboarding additional MN hospital systems
- Expanding this effort to receive electronic lab orders and return results to all birth hospitals in MN

*Specific metrics will be provided.

**Presenter:** Kristen Bye, Minnesota Department of Health, kristen.k.bye@state.mn.us
Objective: Concerns have been raised that screening for congenital hypothyroidism (CH) is leading to excess false positive results and over-identification/treatment. The Minnesota Congenital Hypothyroidism Review project (MNCHR) applied epidemiologic methods to analyze the Minnesota Department of Health’s thyroid-stimulating hormone (TSH) newborn screening data.

Methods: A retrospective review of TSH results and relevant demographic factors was performed including babies born 8/10/16–12/31/20. Prior to analysis, the dataset was cleaned to standardize diagnoses and correct implausible birthweights; without accurate birthweights, incorrect screening algorithms could be applied for result interpretation. Implausible birthweights were defined as 9000 g. Missing birthweights were replaced with birth certificate data when possible. Data were then corrected based on z scores calculated using the MN birth population as a reference. Corrected birthweights < 2000 g were considered low birthweight (LBW).

Case definitions for CH were applied using the first set of clinical thyroid labs prompted by an abnormal screen. Primary CH was defined as high TSH/low free T4 (FT4); subclinical CH as high TSH/normal FT4; normal defined as TSH and FT4 within the normal clinical range.

Results: The dataset included 304374 specimens from 286807 newborns. Prior to data cleaning, 1558 (0.54%) newborns had missing (n=1372) or implausible (n=186) birthweights. Birthweights ranged from 0 to 53297 g and 3.2% were LBW. After correcting the data, only 440 (0.15%) newborns had a missing (n=429) or implausible (n=11) birthweight. Excluding the missing and implausible entries, the birthweights ranged from 300 to 5925 g and 2.4% were LBW.

Applying clinical diagnoses without case definitions, the 4-year prevalence estimate for primary CH was 1:965 newborns screened. Primary CH diagnoses were made five times as often as all other thyroid diagnoses and inaccurately in 78% of the cases. After applying case definitions, the primary CH prevalence estimate decreased to 1:4200 newborns screened.

Implications: MNCHR showed clinical misclassification of primary CH, leading to an inaccurate and drastically elevated prevalence. Using inaccurate diagnoses adversely impacts laboratory test reliability since clinical information is used to evaluate test performance and set analyte cutoffs. Screening programs often use birthweight as a proxy for prematurity and apply different algorithms for LBW infants; missing or inaccurate data can misclassify newborns. While inaccurate birthweights may not be corrected in time for reporting, they should be corrected prior to data analysis or analyte performance evaluation.

This project demonstrates the value of applying epidemiologic methods to newborn screening datasets and shows the necessity for a dedicated epidemiologist on staff in screening programs.

Presenter: Tory Kaye, Minnesota Department of Health, tory.kaye@state.mn.us
Open the Lines of Communication: Increasing Screening Partner Engagement through Improved Quality Reports
H. Winslow, T. Kaye and M. McCann, Minnesota Department of Health, St. Paul, MN

Objective: Engage birth providers in quality initiatives by reporting consistent timeliness and quality metrics on all three parts of newborn screening: blood spot, hearing, and critical congenital heart disease (CCHD).

Methods: In our quest to create an improved newborn screening quality report for all three parts of screening we endeavored to create a consistent reporting schedule with a consistent audience and all-inclusive metrics. We surveyed our hospitals and midwives on improvements they would like to see in our current quality reports. We analyzed feedback, decided which suggestions could be implemented, and created a plan and timeline to test the new statistics and formats. We developed new metrics and format, including a title page and table of contents, an updates page with relevant information about newborn screening and quality, and metrics including timeliness, refer rates, and unsatisfactory rates for each of the three parts of newborn screening. We expanded the audience to include both hospital and midwife birth providers who submit a minimum number of specimens/results within the report period to prevent skewed data for those with a low birth rate.

Results: The first combined newborn screening QA reports were distributed in March of 2021 with overwhelmingly positive feedback from all recipients. Volume and types of feedback from partners increased dramatically. Feedback ranged from updating provider contact information, to asking questions about screening practices and quality metrics, to requesting assistance with process improvement projects. Process improvement projects initiated by partners increased four-fold from 2019 to 2021. Project goals included improving transit time, decreasing hearing referrals, and reducing unsatisfactory blood spot specimens.

Implications: Regular monitoring of data elements at both population and individual facility level can illuminate opportunities for screening improvement. Submitter engagement increases the more you purposefully engage with them with individualized messaging and data. Dedicated data analysts trained in data visualization are therefore important additions to the newborn screening team.

Presenter: Holly Winslow, Minnesota Department of Health, holly.winslow@state.mn.us
You Find What You Look For: Recognizing Patterns in Newborn Screening Data Sets to Engage Partners in QA/QI Initiatives

H. Winslow, T. Kaye, M. McCann, J. Simonetti and S. Rosendahl, Minnesota Department of Health, St. Paul, MN

Objective: Regular monitoring of data elements such as unsatisfactory specimens and transit time is essential to recognizing areas for improvement. We present recent experience engaging blood spot submitters in QA/QI initiatives by providing individualized data visualizations relevant to two projects: 1) responding to a statewide increase in unsatisfactory specimens and 2) improving timeliness by utilizing Saturday deliveries.

Methods: 1) Individual emails were sent to submitters identified with the highest counts of unsatisfactory specimens. We included data visualizations showing statewide and facility-level unsatisfactory metrics and asked that facilities respond back with potential causes for increased unsatisfactory submissions.

2) We performed virtual site visits with facilities to help find ways to improve transit time. A pattern emerged with several facilities not delivering specimens on Saturdays. We identified submitters that had no specimen delivered on a Saturday in 2021, despite collecting specimens on Thursdays and Fridays. We sent targeted messaging to these facilities with instructions to ensure Saturday delivery.

Results: 1) The statewide unsatisfactory percentage increased from an average of 0.44% (2019-2021) to 0.98% in the first quarter of 2022. The first quarter of 2022 showed consistent increases each month beginning at 0.76% in January to 1.1% in March. We identified 24 facilities with increasing trends in unsatisfactory specimens with a median of a 4-fold increase in the percent of unsatisfactory specimens. 15 facilities responded within 1 week with information about filter paper or lancet issues and staffing turnover.

2) Of the 26 facilities contacted about Saturday deliveries, 5 had specimens delivered the first Saturday after contact and 5 more within the next 2 weeks. Overall volume of specimens received and tested on Saturdays continues to increase, resulting in a record number of specimens submitted on a Saturday occurring 3 weeks after contact.

Implications: Regular monitoring of data elements at both population and individual facility level can illuminate opportunities for screening improvement. Addressing increasing unsatisfactory specimen submission prevents delays in valid blood spot screening results and burdensome repeat collections for facilities and families. Utilizing Saturday deliveries decreases the time from birth to result notification by up to 3 days without additional cost or burden to the facility. Submitter engagement increases the more you purposefully engage with them with individualized messaging and data. Dedicated data analysts trained in data visualization are therefore important additions to the newborn screening team.

Presenter: Holly Winslow, Minnesota Department of Health, holly.winslow@state.mn.us
Developing and Updating the ACMG ACTion (ACT) Sheets and Algorithms
M. Lyon, M. Caisse and N. Rose, National Coordinating Center for the Regional Genetics Networks (NCC), Bethesda, MD

Introduction: Since 2004, the American College of Medical Genetics and Genomics (ACMG) has housed the National Coordinating Center for the Regional Genetics Networks (NCC), funded by the Health Resources and Services Administration (HRSA). The NCC develops the ACMG ACT(ion) Sheets and Algorithms. The ACT Sheets are a clinical decision support tool for non-genetic providers on conditions identified through newborn screening. At present, there are 98 Newborn Screening ACT Sheets and Algorithms. In 2021, NCC developed a new process to update and add new Metabolic Conditions Newborn Screening ACT Sheets.

Methods: The ACT Sheets undergo a multi-level review to ensure the information communicated is accurate and useful to non-genetic providers. In 2019, process review noted that many required revision and a systematic method for review and updating ACT sheets was initiated. Following a pilot test of a new development process, a new procedure was implemented to update and add new Metabolic Conditions ACT Sheets and Algorithms. The review process entails:

- Initial Review.
  - A new ACT Sheet is drafted by the NCC Medical Consultant.
  - An updated ACT Sheet and Algorithm is reviewed by a member of the NCC Metabolic Conditions ACT Sheet Small Group.
  - A sub-section of the Small Group reviewed each ACT Sheet and Algorithm to ensure consistent terminology and structure across documents.

- Terminology initially agreed upon by the Small Group.

- NCC Metabolic Conditions ACT Sheet Small Group (membership: genetics providers)
  - All ACT Sheets and Algorithms are:
    - Posted for review by the Small Group.
    - Reviewed together by the Group through an interactive meeting.

- NCC ACT Sheet Advisory Group (membership: non-genetics and genetics providers, family advocate)
  - All ACT Sheets and Algorithms approved by the Small Group are:
    - Posted for review by the Advisory Group.
    - Reviewed together by the Group through an interactive meeting.

- ACMG Board of Directors
  - All ACT Sheets and Algorithms are then reviewed for approval by the ACMG Board of Directors.

At any stage of this process, an ACT Sheet or Algorithm can be sent back to an earlier step if a major comment is made by a member of any group.

Results: As of 5/11, 14 ACT Sheets/1 Algorithms have been updated and 3 new Algorithms have been published. By the summer of 2022, all 24 Metabolic Conditions Newborn Screening ACT Sheets will be updated with the addition of a few new ACT Sheets or Algorithms.

Conclusion: Through this updated development process, NCC ensures the most up-to-date information is presented to individuals who access the ACT Sheets and Algorithms.
**Presenter:** Megan Lyon, National Coordinating Center for Regional Genetics Networks, mlyon@nccrg.org

P-41 is now P-5a
Challenges and Conflicts of the Practical Application of Cystic Fibrosis Case Definitions for Newborn Screening Programs
J. Hale, A. Counihan and A. Comeau, New England Newborn Screening Program, Worcester, MA

Background: Newborn Screening (NBS) programs have a successful history of identifying infants most at risk for conditions and referring them for diagnostic testing. Case definitions for the classification of those identified are vitally important to proper epidemiologic analyses and for evaluations of treatment outcomes and disease progression. We sought to understand the effects of changing classifications of Cystic Fibrosis (CF) cases using our multiyear statewide cohort. We retroactively applied the most recently available case definitions from the Cystic Fibrosis Foundation and Newborn Screening Technical Assistance and Evaluation Program (NewSTEPs) and compared with our own program case definition to better understand inconsistencies and identify data elements that most often contribute to conflicting classifications or insufficient data.

Methodology: Due to its prevalence and wide availability of a standardized diagnostic test, CF provides a model that demonstrates the use and need for well-defined case definitions and the challenges of applying such. We queried our CF database for any child born in Massachusetts in the past 23 years with data suggestive of CF. Specifically, we looked at the NBS results of infants with at least one sweat chloride >=30 mmol/L, including the diagnostic CFTR variants detected and the final determination of whether a CF diagnosis had been established.

Results: There were greater than 700 cases who met the inclusion criteria and required classification. Preliminary comparison between currently published case definitions suggests that up to 10% of CF cases and nearly 50% of CRMS/CFSPID cases might have conflicting case definitions. Much of the CRMS/CFSPID discrepancy is related to evolving case definitions and a lack of clarity for resolution of follow-up. Detailed data and final comparison among three sets of case definitions will be presented.

Conclusions: Harmonization and application of differing case definitions from multiple sources to a large population-based dataset is challenging. Case definitions evolve as new screening and diagnostic technologies become available, generating more data elements to consider. Furthermore, the tracking of changes to an individual’s classification due to more recently obtained diagnostic testing, changing interpretation of a particular genetic variant’s pathogenicity, or clinical judgement, adds challenges. Case definitions for epidemiological purposes can sometimes contradict those needed for clinical purposes. These differences often drive the establishment of multiple case definitions. Awareness among groups of differing definitions/purposes as well as the establishment of a resolution could improve inconsistencies. To understand and monitor which cases are (should be) identified and missed by NBS algorithms, a reliable and consistent case definition is needed.

Presenter: Jaime Hale, New England Newborn Screening Program, jaime.hale@umassmed.edu
**Newborn Screening for the Homocystinurias (Classical and Remethylation Defects) using Methionine (Increased or Decreased) as a Marker: New England Experience**


The New England Newborn Screening Program (NENSP) began screening for Classical Homocystinuria (HCU) in 1968, long before it was included in the Recommended Universal Screening Program (RUSP) in 2009. An increased concentration of Methionine was employed as the marker; in 2015 NENSP expanded the screening panel to include homocystinurias due to defects in the remethylation pathway - Methylene tetrahydrofolate reductase (MTHFR); Cobalamin (Cbl) E, G & Cbl C, D, F defects) by using low methionine concentrations.

The presentation will summarize the NENSP’s experience in the screening for Homocystinuria using Methionine as the primary marker since 1968 – marker distribution, risk assessment and reporting algorithms, modifications over time, screen positive rates, conditions that contribute to the positive screens, true positives and false negatives. The limitations and potential approaches to improve screening with also be reviewed. It is quite likely that even with use of homocysteine ad additional biomarker as first tier, B6 responsive forms may be missed using analyte-based assays alone; targeted scanning for B6 responsive alleles may be necessary to identify this subset. NENSP experience will provide valuable information in screening for the homocystinurias using low methionine concentration as a marker and add to the collective experience of the newborn screening programs in screening for classical homocystinurias.

**Presenter:** Inderneel Sahai, New England Newborn Screening Program, inderneel.sahai@umassmed.edu
P-44

Validation of a Two-tier Testing Algorithm for the Screening of X-linked Adrenoleukodystrophy – The New Jersey Experience
V.R. Floriani, M. O’Neill, P.R. Patel, M.M. Schachter and M.O. Carayannopoulos, New Jersey Newborn Screening Laboratory, Ewing, NJ

Objective: X-linked adrenoleukodystrophy (X-ALD) is a rare metabolic disorder caused by pathogenic variations in the ABCD1 gene. This gene encodes for a protein, ALD, which facilitates the transport of very long chain fatty acids (VLCFA) into peroxisomes for degradation. In patients with X-ALD, this transporter is dysfunctional resulting in the toxic accumulation of VLCFA in tissues including the brain, adrenal glands and spinal cord. New Jersey Newborn screening for X-ALD will employ a two-tiered screening algorithm. The 1st-tier test identifies samples with elevations in C26:0-LPC utilizing the Perkin Elmer NeoBase2 kit via MS/MS (Waters TQD) positive ion mode. Due to a recognized isobaric interferent detected using the positive ion mode, a 2nd-tier test is required. To eliminate the interferent, the 2nd-tier screen employs LC/MS/MS (Waters Acquity UPLC/Xevo) in negative ion mode.

Methodology: In order to verify the performance of Neoobase2 to detect C26:0-LPC - linearity, precision, and accuracy studies were performed following CLSI guidelines. Normal distribution studies were performed by testing 1000 blinded specimens. Based on collaboration with other states and review of the literature, samples with concentrations >97% will be reflexed for 2nd-tier testing. Because it is laboratory developed test (LDT), validation of the 2nd-tier screen requires a full validation including, precision, linearity, accuracy, analytical and diagnostic sensitivity and specificity.

Results: 1st-tier precision was calculated to be within acceptable limits (%CV - PE low 10.4%; PE high 7.0%) based on what was reported in the package insert (%CV – PE low – 13.0%; PE high 9.7%). Using CDC control material, linearity studies were performed and the reportable range verified (0.22 – 2.1 mM). Normal range studies revealed population statistics consistent with those reported in the package insert (New Jersey population mean – 0.26 mM; 99th% = 0.53 mM, 99.5th% = 0.56 mM). Validation of the 2nd-tier test is complete and acceptable performance observed. In preparation for implementation of screening for X-ALD a pilot study was performed to challenge proposed screening cut-offs and optimize laboratory work-flow prior to implementation.

Conclusions: Successful verification and validation of a 2-tiered screening algorithm to detect elevations in the VLCFA C26:0-LPC has positioned New Jersey to implement screening for X-ALD during the 4th quarter of 2022.

Presenter: Victoria Floriani, New Jersey Newborn Screening Laboratory, victoria.floriani@doh.nj.gov
Reclassification of Disease Risk using Psychosine and GALC Genotype in New York Infants Referred for Krabbe Disease
M. Nichols1, C. Saavedra-Matiz1, C. Stevens1, J. Orsini1, R. Wilson1, C. Biski1, D. Wanger2, M. Caggana1; 1Wadsworth Center, New York State Department of Health, Albany, NY, 2Thomas Jefferson University, Philadelphia, PA

Background: The New York State (NYS) Newborn Screening Program (NBS) began screening for Krabbe disease (KD) in 2006 using tandem mass spectrometry (MS/MS) to assess galactocerebrosidase (GALC) enzyme activity (EA). Specimens with GALC EA < 12% of the daily mean were reflexed for second tier molecular testing to identify sequence variants in the GALC gene. Newborns with one or more pathogenic/likely pathogenic or variants of uncertain significance (VOUS) were referred to specialty care centers for diagnostic evaluation. Blood samples were sent to the Lysosomal Diseases Testing Laboratory at Thomas Jefferson University for diagnostic evaluation of GALC EA. Previously, GALC EA was used to classify infants as high-risk (≤ 0.15 µmol/L), moderate-risk (0.16 - 0.29 µmol/L), or low-risk (0.3 - 0.5 µmol/L) for KD. Psychosine (Psy) levels have been shown to be elevated in those who have early infantile (EI) KD. We evaluated whether consideration of Psy levels and GALC genotype along with EA would change the disease risk classification in infants referred for KD in NYS.

Method: Psy levels were determined for 110 infants referred for KD using high pressure liquid chromatography-MS/MS. 29 infants were classified as high-risk for KD based on GALC EA alone. These infants were then reclassified as EI KD, high risk late-onset KD (HRLOKD), low risk late-onset KD (LRLOKD), or unaffected based on Psy levels, GALC EA, and genotype as recommended by Thompson-Stone et al 2021 (PMID:33832819).

Results: Of 29 infants originally classified as high risk for KD, 1 was reclassified as EIKD, 25 were reclassified as HRLOKD and 3 as LRLOKD using the updated KD classification algorithm. Of the 25 infants classified as moderate risk, 2 were reclassified as HRLOKD, 22 as LRLOKD and 1 as unaffected. 43 of the 56 infants (77%) previously classified as low risk were reclassified as unaffected and would no longer require follow-up based on the new recommendations.

Conclusion: Most infants classified as high risk for KD based on GALC EA alone were reclassified as either EIKD or the HRLOKD category resulting in minimal changes to follow-up protocols for these infants. Infants classified as having moderate risk for KD were largely reclassified as LRLOKD using the new algorithm which would allow for less frequent follow-up visits. The most impactful change after implementation of these new risk classification guidelines would be the reclassification of low-risk infants to the unaffected category, where no further follow up would be required. This change is expected to significantly decrease stress on families. The NYS NBS program currently sends samples for all infants referred for diagnostic evaluation to Mayo Clinic for Psy analysis. Results are provided to the specialty care center to be used in conjunction with GALC EA and genotype to assess KD risk.

Presenter: Joseph Orsini, Wadsworth Center, New York State Department of Health, joseph.orsini@health.ny.gov
The Game is Afoot: Using Newborn Screening Tools to Solve Two Cases of Mistaken Identity

As Sherlock Holmes says in Doyle’s ‘A Case of Identity,’ “life is infinitely stranger than anything which the mind of man could invent.” Birth hospitals and attendants submit thousands of newborn screening samples every day. Many procedures within the newborn screening system have become automated, but the process of sample collection and labeling remains a hands-on one that is subject to human error. But if not detected by the staff at the collection site, how can these errors be identified? The New York State Newborn Screening Program (NBSP) reports on two instances of specimen mix-ups which were identified by the Hemoglobin (HgB) lab, confirmed through DNA Identity (ID) testing, and collaboratively researched by Follow-up and hospital staff. In each case, discordant results were initially detected through comparison of an initial specimen’s HgB result to a repeat specimen’s result, a standard procedure used by the NBSP’s HgB lab. In Case #1 the initial sample was collected on DOL 2 and showed AS trait and a borderline TSH, whereas a repeat screen on day 49 showed no S trait and was otherwise negative. These results were confirmed by repunching and retesting the samples. The NBSP’s DNA lab performed ID testing which confirmed the two samples were from different babies, and additionally determined that sample 1 was from a male whereas sample 2 was from a female. Following an investigation, hospital staff determined that sample 1 was likely from a different baby who was in the nursery at the same time. Fortunately, this baby’s pediatrician collected a sample on DOL 20 which identified AS trait. The NBSP did ID testing to compare this sample to sample 1 and confirmed it was the same baby. The hospital’s investigation concluded that an initial screen was not collected prior to discharge for the baby girl from sample 2, so it was fortuitous her pediatrician submitted the “repeat” (her initial). In Case #2, a specimen mix-up was identified in a set of twins by the HgB lab: both twins were identified as having AS trait on their initial screens, but a repeat for Twin A was negative. Both twins were still in the NICU when this was identified, so the NICU was contacted and asked to submit repeat screens for both babies. ID testing confirmed these were from the twins, and that each twin had AS trait, so the aberrant (submitted as Twin A repeat) result was from a third baby. The hospital launched a review that identified a baby who was in the same area of the NICU as the twins the day the discordant sample was collected. ID testing was done to compare a screen known to be from that baby to the discordant sample and it was a match. The HgB lab’s procedure to detect discordant results is uniquely able to detect cases of specimen mix-up. This procedure, coupled with ID testing and old-fashioned detective work, provides a framework for newborn screening programs to detect some of the infinite combinations of errors that may occur.

Presenter: Virginia Sack, Wadsworth Center, New York State Department of Health, virginia.sack@health.ny.gov
Pre-analytical Problems Leading to Post-analytical Woes: Bad Data In, Bad Data Out
M. Caggana, C. Johnson and A. Showers, Wadsworth Center, New York State Department of Health, Albany, NY

The New York State (NYS) Newborn Screening (NBS) Program receives specimens from over 120 birth hospitals and midwives who submit 20,000 specimens per month. As of 2022, 22% of the hospitals submit demographic data electronically. This accounts for 25% of total specimens received. Providers are asked to complete 26 fields on the MCH3 sample collection form via manual or electronic means. Six are critical fields, which are necessary to evaluate results for time critical disorders. Mandatory fields include: 1. date of birth (DOB), 2. date of collection (DOC), 3. time of birth (TOB), 4. time of collection (TOC), 5. gestational age (GA), and 6. birth weight (BW).

In the absence of correct data, NBS laboratories contend with missing demographics by using more conservative or borderline cut-offs likely leading to unnecessary repeat blood draws. If incorrect algorithms are applied due to incorrect or illegible demographics, increased rates of false positive or false negative results lead to poor predictive value and delayed or missed diagnoses.

The NYS NBS Program has undertaken a project to incorporate improvements in a series of queries allowing NBS staff to identify bad demographics in real time. Use of these queries uncovered several specific provider-based data issues. Detection of errors affords staff time to properly interpret results, as the Program has 3 days after receipt to resolve any issues with the 6 critical fields before results are reported.

The poster presents metrics on types of errors received from providers and methods the NYS NBS Program has developed to resolve them.

Presenter: Christopher Johnson, Wadsworth Center, New York State Department of Health, christopher.johnson@health.ny.gov
Second-tier Testing for Congenital Adrenal Hyperplasia (CAH)

J. Dott, D. Kay, N.P. Tavakoli, M. Morrissey and M. Caggana, Wadsworth Center, New York State Department of Health, Albany, NY

Congenital adrenal hyperplasia (CAH) refers to a group of autosomal recessive inherited disorders caused by a deficiency in enzymes involved in cortisol biosynthesis. There is a spectrum of clinical disease severity historically subdivided in three forms: salt-wasting, simple virilizing, & nonclassical. The most common defect, accounting for over 90% of CAH cases, is due to 21-hydroxylase deficiency (21-OHD). The incidence of 21-OHD in the US is estimated to be 1 in 16,000 to 1 in 20,000. 21-hydroxylase is necessary for the conversion of 17-hydroxyprogesterone (17-OHP) to 11-deoxycortisol, leading to the production of cortisol and the conversion of progesterone to deoxycorticosterone, leading to the production of aldosterone. The resulting overload of adrenal androgens and the lack of aldosterone can lead to ambiguous genitalia, adrenal insufficiency, and even death.

All US states, the District of Columbia, and Puerto Rico currently require newborn screening (NBS) for CAH. The primary target for NBS is salt-wasting CAH. Newborns are screened for CAH through measurement of 17-OHP in dried blood spot (DBS) samples, usually by time resolved fluoroimmunoassay. The serum levels of 17-OHP are elevated at birth in infants with CAH, but the rate of false positives may be higher for premature infants or those under 24 hours of age. The NYS Program refers approximately 100 infants and requests repeat samples from >1,000 infants with borderline screens annually, but only 7-11 cases of salt-wasting and simple virilizing CAH are confirmed. The low assay specificity is due to cross-reactivity of the immunoassay antibodies with other steroids.

A method based on liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was shown previously to be effective in reducing the number of CAH false positive results. More recently, the use of four steroids separated by HPLC and two ratios was used as a second-tier test to evaluate NBS samples, resulting in a 95% reduction in the false-positive rate for CAH.

We have evaluated a similar method in New York State. We retrieved approximately 1,000 samples from storage that had been reported as borderline or positive for CAH based on immunoassay. Samples were divided into six groups based on birth weight and age at collection. HPLC-MS/MS results of four steroid markers and two ratios were used to assess samples. The cut-off values were determined by a review of the HPLC-MS/MS results and the clinical diagnosis. For the 1,000 samples we were able to eliminate approximately 85% of requests for a repeat sample and approximately 45% of referrals. The use of second-tier HPLC-MS/MS test for CAH significantly reduces the number of false positive results reported without loss of sensitivity. This can reduce the cost to the medical system and the amount of stress and expense experienced by parents who must wait for confirmatory testing results. Implementation of the assay is planned in 2022.

Presenter: Norma Tavakoli, Wadsworth Center, New York State Department of Health, norma.tavakoli@health.ny.gov
Establishment of Gene Variant Phase from Dried Blood Spots Without Parent DNA: Feasibility and Validation


**Background:** Molecular analysis in newborn screening (NBS) has become more comprehensive, and use of gene sequencing instead of targeted variant panels has already become routine for several NBS Programs. The New York State (NYS) NBS Program performs second- or third-tier gene sequencing for eight conditions using Sanger sequencing, and for cystic fibrosis (CF) a custom next generation sequencing (NGS) assay is used. Comprehensive CFTR gene testing is performed using NGS with deletion/duplication analysis, and only infants with two or more variants are considered screen positive. Single variant carriers are resulted as screen negative. In some cases, referred infants with two or more variants are subsequently found via chromosomal phasing analysis to have inherited all variants from one parent. These infants are CF carriers and not at elevated risk for disease. Without parental samples, phase of identified variants cannot be established. We tested the feasibility of use of drop-phase droplet digital PCR (ddPCR) to infer the cis/trans status of three recurrent CFTR variant pairs.

**Methods:** For each sample, high molecular weight DNA was extracted from two 3-mm dried blood spots (DBS) using a commercial kit. DNA was partitioned into droplets containing reaction mix, primers to amplify both target regions, and labelled hydrolysis probes to hybridize to specific alleles. Four reactions were set up for each variant pair containing primers and probes to target both reference and alternate alleles at each locus. Allele linkage was estimated based on the proportion of alleles co-segregating into droplets. Assays were validated using samples from infants with known phase that were blinded to the analyst.

**Results:** We accurately inferred phase for three variant pairs with 100% concordance with traditional phasing: p.Leu467Phe with p.Phe508del; p.Arg117His with the 5T allele in the polyTG/T repeat region; and p.Ser549Asn with c.*94C>T.

**Conclusion:** Implementation of drop-phase ddPCR testing prior to referral could lead to a reduction in false positive screens, because infants with all variants of potential clinical significance in cis can be released as screen negative. Referral of infants who are carriers and not at risk for disease may lead to parental anxiety, unnecessary testing, and costs, placing increased burden on families, NBS Programs and Care Centers. In NYS, five to six CF referrals could be avoided each year using the three CFTR variant combinations validated thus far, and validation of additional variant pairs for CF and other screened conditions is underway.

**Presenter:** Denise Kay, Wadsworth Center, New York State Department of Health, denise.kay@health.ny.gov
The Impact of a Guanidinoacetate Isobar on Newborn Screening Ontario’s Approach to Guanidinoacetate Methyltransferase Deficiency Screening

N. McIntosh, D. Durie, E. Desormeaux, A. Milks, P. Chakraborty, N. Lepage and M. Henderson, Newborn Screening Ontario, Ottawa, ON Canada

Objectives: Guanidinoacetate Methyltransferase (GAMT) deficiency is a rare autosomal recessive disease in which the GAMT enzyme, responsible for the conversion of guanidinoacetate (GUAC) to creatine (CRT), is deficient resulting in elevated GUAC and deficient CRT. CRT and ornithine supplementation is an effective treatment, but when left untreated neurological manifestations present in infancy, and autistic behaviors in older children. GAMT newborn screening (NBS) involves, multiplexing GUAC and CRT with amino acids/acylcarnitines (AAAC) flow injection (FIA) MS/MS screening assays. In derivatized FIA-MS/MS assays, a GUAC isobar, sharing the MRM transition 174>101, causes falsely elevated GUAC results. Use of a second-tier LC-MS/MS assay provides chromatographic resolution of GUAC and the isobar. Alternatively, the use of a different MRM transition 174>73 with FIA-MS/MS has shown to be more specific to GUAC. Here we present the impact of the isobaric interfering compound on the method development of GAMT assays by Newborn Screening Ontario (NSO).

Methods: First-tier FIA-MS/MS
GUAC was multiplexed with the current butylated AAAC assay by adding 13C2-GUAC internal standard (IS) to the extraction solution and inclusion of 174>101 and 174>73 transitions for GUAC and 176>103 for GUAC-IS.

Second-tier LC-MS/MS
Single 3.2mm DBS samples are processed in a 96 well plate, multiplexing GUAC with total homocysteine (HCY). The acetonitrile/H2O based extraction solution contains dithiothreitol, GUAC-IS and HCY-IS. This assay uses butylation and the first-tier GUAC and GUAC-IS transitions indicated above.

Results: Precision data from 4 DBS samples with spiked GUAC of 0, 5, 10 and 20 µmol/L produced CVs of < 8% at each level for both FIA-MS/MS and LC-MS/MS (n=30).
n=206 DBS samples were analyzed by FIA-MS/MS and LC-MS/MS. Elevated (174>101/174>73) ion ratios on FIA-MS/MS predicted samples with isobar interference. Correlation of FIA-MS/MS and LC-MS/MS GUAC quantitation was performed using three FIA-MS/MS data sets against LC-MS/MS using the GUAC 174>101 transition. Poor agreement was observed when all samples were included using GUAC 174>101; Deming regression 0.26 + 0.57 * 1st tier (n=206). Good agreement was observed when using GUAC 174>73; Deming regression 0.167 + 0.99 * 1st Tier (n=206); and when using GUAC 174>101 if samples with ion ratios >4 were excluded; Deming regression 0.07 + 0.99 * 1st Tier (n=153).

Conclusions: The GUAC isobar is an important consideration when NBS programs implement GAMT screening. The use of GUAC 174>101 quantitation by FIA-MS/MS necessitates a second-tier LC-MS/MS assay to resolve GUAC from the isobar to reduce false positives. Alternatively, the use of GUAC 174>73 for quantitation by FIA-MS/MS eliminates the isobar impact and makes LC-MS/MS less important. NSO will continue to investigate both options while working towards implementing GAMT screening in Ontario.

Presenter: Nathan McIntosh, Newborn Screening Ontario, nmcintosh@cheo.on.ca
Critical Congenital Heart Disease (CCHD) refers to a group of serious congenital heart defects that affect the structure of the heart and will require surgery or catheter intervention within the first year of life. CCHD prevents the heart from pumping blood effectively or reduces the amount of oxygen in the blood, both of which can lead to organ damage or result in life-threatening sequelae. Congenital heart disease has an incidence rate of 12 per 1000 births, and of those, 25% are critical cases. The current standard approach to detect CCHD includes three clinical tools: prenatal ultrasound, physical exam and pulse oximetry screening. Pulse oximetry screening can identify infants with CCHD before the presentation of symptoms, resulting in better outcomes for the infant.

Newborn Screening Ontario (NSO) is the provincial program that coordinates newborn screening in Ontario. In 2017, NSO began implementing point of care screening for CCHD through using pulse oximetry throughout the province.

The program evaluation sought to provide a better understanding of the success of implementation strategies, current algorithms and their appropriateness; characteristics of infants identified prenatally or by physical exam; characteristics of infants who have false positive and false negative screens; and the sensitivity and specificity of the algorithms used.

Over the two and a half year period of data collection, there were 533 screen positive infants (27 primary targets, 98 secondary targets, 87 incidental targets and 321 false positives) in 327,169 screened infants. There were 4 reported false negatives for primary targets and 3 false negative secondary targets. There was a significant difference among males and females for false positive screens; males were significantly more likely to screen false positive compared to females ($p = .053985$). The sensitivity of pulse oximetry screening in Ontario is 79.4% for primary targets and 94.7% for primary and secondary targets. There were 580 missed screens; one tertiary care hospital accounted for 146 of these missed screens and were infants who would not have been suitable for screening. Overall, the screening program was well received by healthcare providers and implementation went relatively smoothly. Based on the results of the evaluation, the following recommendations have been made:

- Conduct further research to understand maternal and fetal risk factors
- Emphasize the importance of CCHD screening to detect non-cardiac issues
- Develop multilingual pamphlets to be given to parents to improve and standardize the educational material that they receive

**Presenter:** Bailey Milne, Newborn Screening Ontario, bmilne@cheo.on.ca
Implementation of Newborn Screening for X-Linked Adrenoleukodystrophy in North Carolina
K. Blake, J. Mills, S. Freeman, D. Pettit and S. Shone, North Carolina State Laboratory of Public Health, Raleigh, NC

Objective: The North Carolina State Laboratory of Public Health MSMS Laboratory implemented newborn screening for X-Linked Adrenoleukodystrophy (X-ALD) in February 2022. The performance of the NeoBaseTM2 Non-Derivatized MSMS kit on PerkinElmer Qsight 225 MD UHPLC Screening Systems was verified to measure C26:0-lyosphosphatidylcholine (C26:0-LPC) as a 1st-tier method. The performance of a negative ion mode LC-MS/MS Laboratory Developed Test on SCIEX Citrine™ QTRAP MS/MS was validated to measure C26:0-LPC as a 2nd-tier method.

Method: The 1st-tier method verification included studies for accuracy, precision, reportable range, carryover, instrument comparison, and reference range for cutoff establishment. Precision was determined by analyzing Kit QC and CDC QC over 5 days. Population-based cutoffs were established by analyzing 5,125 patient specimens to identify the concentration to reflex to the 2nd-tier method.

The 2nd-tier method validation included analyses of accuracy, precision, reportable range, sensitivity, carryover, specificity, interferences, stability, and cutoff establishment. Precision was determined by analyzing CDC QC over 5 days. A population study of 2,581 specimens was conducted to establish the cutoff for normal and abnormal interpretations.

Accuracy for both methods was determined by analyzing 10 historical CDC PT specimens, 11 known specimens, and 35 blind samples.

Results: Precision studies, including intra-run, inter-run, inter-day, and inter-operator, showed an average %CV of < 10% for C26:0-LPC for both the 1st-tier and second-tier methods.

All known specimens in the 1st-tier accuracy study were above the established fixed cutoff of ≥0.40 µM except for 2 known female carriers. The accuracy data were also evaluated using a floating cutoff which reflexed the top 3% of specimens on each plate for 2nd-tier testing. This allowed for the identification of the female carriers.

The 2nd-tier cutoff was set at 99.93 percentile of the population data gathered. Using this cutoff, all known specimens had consistent interpretations along with 24 out of 25 blind specimens. The single discrepancy was a specimen previously characterized as normal; but was abnormal when using the established 2nd-tier cutoff. C26:0-LPC was determined to be stable in DBS specimens that were stored at ambient temperature and humidity for over 2 weeks with no observed interferences.

Conclusions: Approximately 20,000 specimens have been screened using a 3% floating cutoff; reflexing 10% for 2nd-tier testing to identify 4 female specimens with elevated risk for X-ALD. Confirmatory testing using VLCFA analysis and ABCD1 gene testing and follow-up counseling determined 1 case to be a false positive and 1 case as having a known family history of X-ALD. Two cases are pending ABCD1 gene testing.

Presenter: Kimberly Blake, North Carolina State Laboratory of Public Health, kimberly.blake@dhhs.nc.gov

P-53 – Withdrawn
Improving Long-term Follow-up (LTFU) in a Newborn Screening (NBS) Program
A. Patterson¹, J. Baysinger²; ¹University of Oklahoma Health Science Center, Norman, OK, ²Oklahoma State Department of Health, Oklahoma City, OK

In the spring of 2022, the Oklahoma NBS program manager acted as the preceptor and mentor for a Masters in Nursing Administration graduate student's final project. The project focused on analyzing and reviewing their current LTFU program and researching ways to improve the program. Research included collaborative meetings with seven other NBS LTFU state programs as well multiple other key stakeholders involved in NBS nationwide. Though key features of a LTFU program were defined by the United States Secretary of Health and Human Services' Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children (ACHDGDNC) in April of 2007 (Kemper et al., 2008), LTFU programs are still lacking across the country with recent survey data collected in 2020 from 33 state programs showing 41% are not currently utilizing any form of LTFU in their program (Darby et al., 2021). There continues to be no standard guidelines or benchmarks for programs to be following. As disorders with more complex treatments continue to be added to the recommended uniform screening panel, the need for care coordination and data collection beyond diagnosis through LTFU is necessary to track program benefits and long-term outcomes (Darby et al., 2021). In addition to improved tracking, a LTFU program with clear guidelines and goals has the potential to close many of the gaps currently faced in NBS programs.

This poster presentation will offer a summative report on the findings found throughout the research project including the proposed improvement plan. The presenters hope this presentation can help cultivate conversations with other NBS programs regarding how to initiate a LTFU program or improve their program further, as well as create consistency across NBS programs nationwide

**Presenter:** Jennifer Baysinger, Oklahoma State Department of Health, jenniferxa@health.ok.gov and Amanda Patterson, University of Oklahoma Health Science Center, amanda.patterson@integrisok.com
Determination of Mucopolysaccharidosis Type II (MPS II) Enzymatic Activity in Dried Blood Spots using the PerkinElmer QSight® 225MD UHPLC Screening System

Mucopolysaccharidosis type II (MPS II), also known as Hunter syndrome, is a lysosomal storage disorder caused by a deficiency in the iduronate-2-sulftase (I2S) enzyme. The I2S enzyme is responsible for degradation of glycosoaminoglycans (GAGs). In the disease state accumulation of GAGs is observed, leading to clinical pathogenesis, including stunted growth, joint deformities and significant mobility issues. Treatments such as enzyme replacement therapy are available, speaking for the need to screen for I2S activity in the early days of life. Herein, we describe a research use only (RUO) product for monitoring I2S activity in dried blood spots (DBS) coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS). We demonstrate measurement of I2S activity across a wide range of activities by incubating the DBS with a surrogate substrate and monitoring enzyme product formation. A chromatography-based approach is used due to the propensity of the substrate to fragment into product within the ion source. In this study, we analyze a population of neonate samples against leukocyte-depleted dried blood spots with low I2S activity, demonstrating the dynamic range of enzymatic activity. The PerkinElmer QSight® 225MD UHPLC screening system screening system proved to be an effective and sensitive solution for screening I2S activity, when coupled with the enzymatic assay described herein.

Presenter: Collin Hill, PerkinElmer, collin.hill@perkinelmer.com
Measurement of Lysophosphatidylcholine (26:0) in Dried Blood Spots via a Second-tier LC-MS/MS Assay using the PerkinElmer QSight® 225MD UHPLC Screening System

C. Hill, J. Trometer, R. Korathu and M. Giolito, PerkinElmer, Waltham, MA

Lysophosphatidylcholine (26:0) also known as LysoPC(26:0) is a biomarker for the rare disease x-linked adrenoleukodystrophy (XALD). In the disease state, very long-chain fatty acids (VLCFAs) such as LysoPC(26:0) accumulate and lead to the progressive loss of protective myelin surrounding the nerves of the brain and spinal cord. Certain treatments, such as supplementation with Lorenzo’s oil may help treat the disease, speaking to the need for screening in the early days of life. While a rapid tier 1 test for LysoPC(26:0) is available from the PerkinElmer NeoBase™ 2 MSMS kit, tier 2 assays are typically performed for confirmation. Herein, we describe a research use only (RUO) product for determination of LysoPC(26:0) concentrations in dried blood spots using liquid chromatography tandem mass spectrometry (LC-MS/MS). In this study, dried blood spots spiked with varying concentrations of LysoPC(26:0) are assayed using a quick 30 minute incubation and subsequent analysis on a PerkinElmer QSight® 225MD UHPLC screening system. The assay may also be multiplexed to monitor other VLCFAs, such as LysoPC(24:0). This workflow demonstrates a rapid, robust and sensitive solution for determination of LysoPC(26:0) in dried blood spots via a second tier assay.

Presenter: Collin Hill, PerkinElmer, collin.hill@perkinelmer.com
DryPCR in Detecting the Absence of SMN1, TREC and KREC from Extracted DNA from DBS
M. Siitonen, T. Helenius, H. Savela, M. Makinen, I. Alm-Ndiaye, V. Veikkolainen, M. Aaltoranta and M. Hjort, PerkinElmer Wallac, Turku, Finland

PerkinElmer has recently developed a ‘for research use only’ dry qPCR assay for the qualitative detection of the homozygous deletion of survival of motor neuron 1 (SMN1) gene exon 7 and the quantification of T-cell receptor excision circles (TREC) and kappa-deleting recombination excision circles (KREC). This assay can run up to 96 samples in approximately 2 hours from a dried blood spot sample (DBS). The Eonis™ SMN1, TREC, KREC assay uses dried blood spot (DBS) samples and consists of a short 2-step DNA extraction protocol and sample addition to the PCR plate, and therefore does not require any dedicated clean room or freezer space.

The Eonis™ SMN1, TREC, KREC kit detects qualitatively the absence of exon 7 in the SMN1 gene simultaneously with the quantitative detection TREC and KREC copy numbers. The multiplex assay utilizes ribonuclease P/MRP subunit P30 (RPP30) as an internal amplification control, to monitor the quality of the extracted DNA. The assay is monitored using SMN1/TREC/KREC-positive and SMN1/TREC/KREC-negative DBS controls, which are extracted and processed simultaneously with the samples throughout the whole workflow. The Eonis™ SMN1, TREC, KREC kit is developed together with the Eonis™ Q PCR cycler, making up a robust, effective, and easy to use total solution consisting of both reagents and instruments.

Regardless of research lab size and resourcing, the Eonis™ SMN1, TREC, KREC kit is an elegant, simple, and effective research method for the detection of SMN1, TREC and KREC absence in extracted DNA. Please check with your local representative for availability.

For research use only. Not for use in diagnostic procedures.

Presenter: Terhi Helenius, PerkinElmer Wallac, Terhi.Helenius@perkinelmer.com
Validation of an LC-MS Dried Blood Spot Enzyme Assay for the Screening of Mucopolysaccharidosis Type II

S. Blake¹, V. Robles¹, B. Migliore³, J. Apoian², S. Young², E. Jalazo³, D. Bali², K. Clinard¹, K. Blake⁴, D. Pettit³, J. Carter¹, H. Bilbrey³, L. Torrice³, S. Shone⁴, C. Rehder⁷, J. Muenzer³, L. Gehtland¹, M. Raspa¹, K. Kucera¹; ¹RTI International, Research Triangle Park, NC, ²Duke University, Durham, NC, ³University of North Carolina at Chapel Hill, Chapel Hill, NC, ⁴North Carolina State Laboratory of Public Health, Raleigh, NC

Mucopolysaccharidosis II (MPS II) is a rare X-linked lysosomal storage disorder affecting approximately 1 in 100,000 babies. Patients with MPS II can have a spectrum of clinical involvement with somatic disease and cognitive impairment (neuronopathic or severe form) to only somatic disease (non-neuronopathic or attenuated form). Patients with MPS II usually appear normal at birth with signs and symptoms of disease typically appearing after 1 to 2 years of age in the severe form. Presenting features include hernias, hearing impairment, recurrent infections, liver and spleen enlargement, coarse facial features, decreased joint range of motion and delayed development. About two thirds of MPS II patients have the neuronopathic form and if untreated die typically in the 2nd decade of life. Diagnosis of MPS II is confirmed by deficient iduronate-2-sulfatase (I2S) enzyme activity and accumulation of glycosaminoglycans (GAG) in urine. Early detection of MPS II patients prior to onset of significant clinical disease will provide opportunity to start treatment early and the potential to minimize the occurrence of irreversible organ damage.

We report on the validation of an MPS II screening assay in North Carolina. The MPS II screening assay is a laboratory-developed test for I2S enzyme activity in blood. Newborn dried blood spots (DBS) are incubated with a cocktail solution containing I2S enzyme substrate and internal standard (IS). After incubation, the samples are extracted, dried, and reconstituted in mobile phase then analyzed via positive-ion LC-MS/MS. The peak area ratio of the I2S-product and IS are used to calculate enzyme activity.

This validation assessed the I2S enzyme activity in dried blood spots to determine analytical specificity, sensitivity, linearity, precision, carryover, and stability of reagents and enzyme activity in DBS. In addition to these tests, a retrospective screen of ~6,000 de-identified NBS specimens from North Carolina newborns was performed to establish an initial reference range specific to the screened population. The initial accuracy of the cut-off established in the population study was also tested. This validation was performed to prepare for a pilot study that will screen ~140,000 newborns in North Carolina.

Presenter: Samantha Blake, RTI International, slblake@rti.org
Does a Telephone Reminder After Receipt of a Mailed Recruitment Letter Impact Enrollment in a Newborn Screening Research Study?


Recruitment of participants is a challenge for many research studies. Letters mailed to the physical addresses of potential participants are a common recruitment method; however when used alone, mailed recruitment letters are often not sufficient to achieve target study enrollment. Some studies have found that a telephone call made to a potential participant after a mailed recruitment letter may increase study enrollment. In this manuscript, we report on enrollment in the Early Check newborn screening research study among a group of new mothers who were randomly assigned to receive a personal telephone reminder shortly after a mailed recruitment letter, compared to a control group of new mothers who received a letter only. The experiment began in November 2021. Interim analysis of data collected through February 2022 found that within the reminder call group, 52.4% of women were left the telephone reminder as a voicemail; 15.6% received the telephone reminder in full as a personal telephone conversation; and 32.0% could not be reached, had incomplete calls, or were ineligible. An interim analysis showed that women were more likely to enroll if they had a completed call or were left a message, compared to women who received a letter-only. Additionally, an interim intent to treat analysis of the two randomized groups found significantly greater enrollment rates among women in the letter-plus-telephone-reminder group (5.2%), regardless of the outcome of the call, compared to the letter-only group (4.6%), \( \chi^2 (1, N = 21,263) = 4.39, p = .036 \). In this poster we will report on the final analysis using data between November 2021 and June 2022.

Presenter: Lisa Gehtland, RTI International, lgehtland@rti.org
Early Check Newborn Screening for Angelman, Prader-Willi, and Dup15q Syndromes: Assay Preparation and Validation
B. Migliore¹, K. Kucera¹, A. Wheeler¹, E. Jalazo²; ¹RTI International, Research Triangle Park, NC, ²University of North Carolina at Chapel Hill, Chapel Hill, NC

Angelman syndrome (AS), Prader-Willi syndrome (PWS), and Dup15q syndrome are largely caused by copy number variants and aberrant inheritance of imprinting markers at the 15q11-13 locus resulting in inappropriate DNA methylation patterns and disruption of gene regulation. The conditions have different etiologies but share diagnostic options and are targets for emerging therapeutics. The average age of diagnosis ranges from 6 months for infants with PWS to 3 years for children with Dup15q, and much later for cases of nondeletion subtypes of PWS or AS (Wheeler, A.C, et al in preparation). Newborn screening (NBS) provides the only population-based strategy to identify infants who could benefit from early disease detection and treatment; however, the lack of an affordable and accurate screening test for presymptomatic identification of babies with PWS, AS and Dup15q has been a limitation. Early Check, an ongoing expanded NBS study currently taking place in North Carolina, provides an opportunity to generate data on early identification of individuals with rare disorders and inform public policy.

A major challenge in adding screening for PWS, AS and Dup15q to states’ NBS panels will be the establishment of a new testing methodology in state laboratories to detect the specific methylation patterns at the SNRPN/UBE3A locus that are the molecular signatures of these conditions. Since detection of methylation changes requires additional procedural steps prior to molecular testing, demonstrating the feasibility of these procedures, in addition to evaluating the performance of the assay, to the current molecular NBS workflows will be essential for implementing state-wide screening. We will present the necessary steps that are being performed to add screening for PWS, AS and Dup15q to Early Check with focus on evaluating a candidate screening assay [Godler et al., 2022 PMID 34982160]. The quantitative melt analysis (MS-QMA) is a low cost methylation-specific first-tier test for use with dried blood spots (DBS) to detect abnormal levels of SNRPN promoter methylation. The test involves sample lysis to release DNA, automated bisulfite conversion, real-time PCR detection, and methylation ratio analysis to detect methylation signatures specific to each disorder. We will describe the testing workflow and steps to implementation and validation of the assay to assess precision, accuracy, analytical sensitivity, and reference intervals performed prior to implementation in Early Check for prospective NBS. Further, we will discuss adaptation of Early Check materials and processes including public outreach materials, IRB approvals, research portal updates, development of PWS, AS and Dup15q -specific educational content, condition-specific informatics and quality assurance, diagnostic confirmation, and short-term and long-term follow-up protocols.

Presenter: Brooke Migliore, RTI International, bmigliore@rti.org
Using a Team Science Approach to Develop Short-term Follow-up Protocols and Educational Materials

M. Raspa1, L. Percenti2, M. Fort2, J. Watkins3, K. Blake3, B. Wright1, K. Kucera1, M. Sontag4, S. Shone3, Y. Kellar-Guenter4; 1RTI International, Research Triangle Park, NC, 2North Carolina Division of Child and Family Well-Being, Raleigh, NC, 3North Carolina State Laboratory of Public Health, Raleigh, NC, 4Center for Public Health Innovation, Littleton, CO

Problem: Creating short-term follow-up protocols and education materials is an important step to ensure families get timely diagnostic testing and treatment to maximize the benefits of newborn screening (NBS). Ideally, these processes involve follow-up program staff, clinical specialists, and NBS laboratory staff (Kellar-Guenther et al., 2020). Yet, NewSTEPs site review reports highlight the need for stronger communication between the laboratory, follow-up, and specialists (Kellar-Guenther et al., 2019).

Objectives: With funding from the CDC, North Carolina (NC) is adding four new disorders to their newborn (NBS) screening panel. Because these conditions are complex in their clinical presentation, a more collaborative process for short-term follow-up was needed. We piloted a team science approach which included state NBS follow-up staff, clinical specialists, and laboratory staff to create protocols and educational materials for each condition.

Methodology: Prior to this project, the NC follow-up team created protocols and education materials and gathered input through email from the specialists. The laboratory was not involved and did not provide insight into the screening approach. For this project, state NBS follow-up staff, clinical specialists for each condition, and NBS laboratory representatives--met bi-weekly over ZoomTM to create the needed materials prior to the condition being added to the NC screening panel. After screening for each condition began, the follow-up team and laboratory staff met monthly with specialists across the state to discuss screen-positive cases and determine if changes needed to be made to the protocol or education materials.

Results: The team science approach fostered an inclusive environment in which clinical specialists felt more involved in the process of on-boarding new conditions. The use of videoconferencing enabled the team to work on the protocol or education material in real time and share feedback immediately. Specialists also felt better informed on the laboratory process for adding a new condition and the timing of when screening would begin. In addition, the laboratory staff gained a better understanding of the complexity of clinical follow-up. Finally, the team was able to create a template of what needed to be included in parent and provider education materials, which made it easier to organize critical information and create a similar structure across conditions.

Conclusions: The use of a team science approach, which was facilitated through videoconferencing, enabled the multidisciplinary team members in different departments/organizations to participate in the planning of short-term follow-up. This approach has enabled shared learning across the NBS program, increased communication and collaboration, and the development of high-quality resources that are tailored to NC.

Presenter: Melissa Raspa, RTI International, mraspa@rti.org
Future Collaboration Between Early Intervention and Newborn Screening
S. Andrews¹, S. Blanchard², P. Chakraborty³, A. Isiaq¹, E. Jalazo⁴, S. Scott¹, D. Bailey¹; ¹RTI International, Research Triangle Park, NC, ²East Carolina University, Greenville, NC, ³Newborn Screening Ontario, Ottawa, ON Canada, ⁴University of North Carolina at Chapel Hill, Chapel Hill, NC

Objectives: Newborn screening (NBS) identifies children with specific medical conditions that benefit from early detection and treatment. However, even after available treatment, many children experience delays and could benefit from Early Intervention (EI) services. The current presentation integrates findings from two studies examining the links between EI and NBS. Specifically, we will describe whether NBS refers children to EI after a NBS diagnosis and whether NBS provides information to parents on EI after an NBS diagnosis. Next, we describe whether there is an available, consistent list of conditions that should be referred to EI. Lastly, we report what NBS conditions should be referred to EI because they have a high probability of a delay. We propose opportunities for future collaboration between EI and NBS.

Methods: To understand whether NBS programs are referring children to EI or whether they are providing information and resources on EI to families, all state NBS coordinators were asked to complete an online survey. Each state’s EI eligibility criteria was examined to determine which NBS conditions are auto-qualified. Lastly, we developed a matrix to assess risk of delay in treatment-altered natural history, extent of medical complexity, and likelihood of episodic decompensation for each NBS condition. After extensive literature reviews, two NBS experts independently classified each condition on the matrix.

Results: Most NBS coordinators disagreed that NBS program and/or long term follow-up staff are making the referral of children diagnosed with a condition through NBS for EI services. Similarly, most NBS coordinators disagreed that NBS program staff was providing families with resources and information on EI. We also found there is not an available, consistent list of conditions that are auto-eligible for EI. The average number of NBS conditions that auto-qualify for EI is 7.6. Our literature review and a consensus process demonstrated that 29 RUSP conditions likely meet national criteria to be an EI auto-qualified condition.

Conclusions: NBS is a universal program. There is a unique opportunity to locate and evaluate more children who have a high probability of developmental delay. NBS could be designated as a Child Find Resource to ensure all appropriate children are identified, located, and evaluated for EI. However, NBS can only refer appropriate children if there is clarity and guidance regarding which conditions should qualify for EI. Our results suggest most NBS conditions should automatically qualify based on probability of delay. These findings suggest a future opportunity for collaboration between NBS and EI programs to create a consistent set of auto-qualified conditions, potentially expedite referrals of eligible children, and streamline children’s access to EI services.

Presenter: Elizabeth Reynolds, RTI International, erreynolds@rti.org
Newborn Screening for Congenital Hypothyroidism & Screen Positive Exome Sequencing Among Neonates in a Tertiary Care Centre in Pondicherry, India – A Cross Sectional Study

V.B. Sugumaran¹, S. Sumathi², K. Karthikeyan²; ¹Saveetha Medical College & Hospital, SIMATS, Pondicherry, India, ²MGMCri, SBV, India

Introduction: Newborn Screening (NBS) is screening in neonates between 48 – 72 hours of life for conditions that are treatable, but not clinically evident in the newborn period. NBS was first started in 1961 by Robert Guthrie, and is considered to be one of the greatest public health achievements. The goal is to identify infants at risk for several conditions early enough to confirm the diagnosis and provide intervention that will alter the clinical course of the disease and prevention of further clinical manifestations. It is important to estimate the incidence of treatable disorder like congenital hypothyroidism (CH), as there is paucity of data, from Indian population, conducted on large sample size. By conducting a prospective cross sectional study, we aim to identify the prevalence of the disorder in this region.

Materials & Methods: All neonates who were born between June 2021 and Apr 2022 at the study centre are included in this cross-sectional study. Babies admitted to the Neonatal Intensive Care Unit (NICU) were screened between 48 h of life and prior to discharge, were offered screening after initial stabilization.

Three drops of capillary blood are collected on 903 S filter paper through heel prick method. The dried blood samples are routed through the hospital’s central laboratory to the newborn screening laboratory – Apollo Health and Lifestyle Limited, Secundrabad. Neonates discharged before 48 h, those who died in the NICU and those babies whose parents refused consent did not undergo NBS.

Thyroid Stimulating Hormone (TSH) for diagnosing Congenital Hypothyroidism was assayed by Flouroenzymatic Immunoassay with interpretaion as 20: High Serum TSH. For screen positive samples Thyroid profile including fT3, fT4, TSH were performed and those positive cases were taken for Exome sequencing.

Results: The results and discussion will be revealed at time of oral presentation.

Presenter: Vinod Babu Sugumaran, Saveetha Medical College & Hospital, drvinodbabu@gmail.com
Comparative Analytical Performance of the Next-Generation Sebia CAPILLARYS 3 DBS Instrument for Newborn Hemoglobinopathy Disorder Screening

C. Williams\textsuperscript{1}, M.C. Dorley\textsuperscript{2}, T. Childs\textsuperscript{2}, K. Anderson\textsuperscript{1}, J. O’Leary\textsuperscript{1}; \textsuperscript{1}Sebia, Atlanta, GA, \textsuperscript{2}Tennessee Department of Health: Division of Laboratory Services, Nashville, TN

**Background:** The Tennessee Department of Health Division of Laboratory Services Newborn Screening Laboratory (TN Lab) screens over 85,000 newborns each year for variant hemoglobins (Hb) while under operational constraints to including staffing and budget. TN Lab also serves an increasingly diverse population driving the need to identify Hb variants accurately and precisely to ensure a high-level of patient care. With these challenges in mind, the TN Lab conducted a method comparison study measuring analytical performance between three platforms: HPLC method (Bio-Rad VARIANT nbs), previous generation Capillary Electrophoresis (CAPILLARYS 2 NEONAT Fast [Sebia]), and the next-generation CAPILLARYS 3 DBS instrument (Sebia).

**Methods:** The TN lab screened 393 newborn dried blood spot specimens between August – October 2021. These specimens covered a broad range of ethnic diversities, age (hours), weight, and Hb variant status. The specimens were analyzed concurrently on the three platforms and the Hb patterns were compared and analyzed for equivalence.

**Results:** For the 393 specimens, there was 100% correspondence between the predicate CAPILLARYS 2 NEONAT Fast and the CAPILLARYS 3 DBS in identification of normal/pathological results. Of the 393 specimens, 53.4\% (n=210) were identified as normal, with 46.6\% (n=183) pathological specimens detected. FAS (34.97\%), FAC (19.67\%), FA+Bart’s (19.13\%), and FAS+Bart’s (6.56\%) were the predominant hemoglobin variants detected. The CAPILLARYS 3 DBS separated Hb D (D-Punjab / D-Los Angeles) from Hb G-Philadelphia in six patients compared. It also detected prevalence of Hb Barts in >26\% of patients.

**Conclusions:** In this study, Sebia’s CAPILLARYS 3 DBS system demonstrated equivalent clinical performance compared to the previous generation CAPILLARYS 2 NEONAT Fast for newborn screening of Hb variants. Additionally, compared to existing methods, Sebia’s Capillary Electrophoresis separation has the resolution to distinguish common Hb variants (including S, C, D, E, Bart’s and G-Philadelphia, among others) for simplified analysis and reporting in the Sebia Phoresis software. The CAPILLARYS 3 DBS system’s automated workflow, high specimen throughput, combined with high-resolution clinical results presents an exciting technological advancement for newborn Hb variant screening.

**Presenter:** M. Christine Dorley, Tennessee Department of Health, m.christine.dorley@tn.gov

**P-65 – Withdrawn**
**Implementation of Weekday Courier Service for Newborn Screening Specimens**

H. Davis-Martin and B. Bair, South Carolina Dept of Health & Environmental Control, Columbia, SC

Newborn screening (NBS) quality improvement (QI) plays an integral role in the South Carolina (SC) newborn screening program. The primary focus of NBS QI is to improve the quality of blood spots collected and reduce the turnaround time for blood spot specimens from SC birthing hospitals and pediatrician offices to the SC Public Health Laboratory (PHL). To achieve these goals, NBS QI staff regularly train hospital staff on specimen collection, packaging, and shipping with the goal of getting specimens to the PHL as quickly as possible to improve health outcomes for SC newborns.

In 2019, the SC PHL NBS Program received funding from the Association of Public Health Laboratories (APHL) for a Continuous Quality Improvement (CQI) grant to improve turnaround times for NBS specimens by implementing a dedicated courier service for NBS specimens on Sunday evenings. The SC PHL NBS Program saw immediate improvement in specimen turn-around-time (TAT) using a dedicated service one day a week and began the implementation of an additional dedicated courier service to pick-up specimens Monday through Friday. The weekday courier service was implemented for SC hospitals in September 2021. The SC PHL NBS Program utilizes two private courier services that pick-up NBS specimens from SC hospitals Sunday through Friday evenings starting at 9:00 pm for delivery to the PHL before 7:00 am the following day.

Currently, 37 out of 38 SC birthing hospitals are participating in the Sunday and weekday courier service. To date, the weekday courier service has improved turnaround times by an average of 24.3% across all SC hospitals. Additionally, 93% of SC hospitals are seeing at least a 5% decrease in TAT compared to the previous year.

**Presenter:** S. Graham McCaskall, South Carolina Dept of Health & Environmental Control, mccasksg@dhec.sc.gov
Newborn Screening Dashboard: A Tool for Improving Tennessee Newborn Screening Quality

C. Lechner¹, M. Rumpler¹, M.C. Dorley¹, Y. Li², A. Ingram², H. Fryman²; ¹Tennessee Department of Health: Division of Laboratory Services, Nashville, TN, ²Tennessee Department of Health: Division of Family Health and Wellness, Nashville, TN

Objective: The Tennessee Newborn Screening (NBS) Program has recently released a public Tableau dashboard to visualize dried blood spot (DBS) screening performance data across TN. To better drive NBS quality improvement, the NBS program is proactively evaluating measures to identify areas that need corrective action based on data and observations from this external dashboard, as well as other dashboards currently under development.

Methodology: On May 2, 2022, the NBS Dashboard was officially launched as the result of a collaboration between the TN NBS Program and a fellow from APHL’s NBS Bioinformatics Fellowship. This dashboard visualizes trends of DBS screening rate, unsatisfactory DBS rate, and the timeliness of DBS collection, transportation, and reporting of results at the state and individual facility level. Using the dashboard, NBS staff identified past instances of NBS performance that significantly deviated from expected performance goals, investigated them to ascertain the root cause and corrective actions that were or could have been taken, and the effectiveness of such actions. A Plan-Study-Do-Act approach was used to address identified issues and procedures were updated, or developed, to minimize future issues identifiable through data trends on the dashboard. Additional dashboards with more performance indicators are under development and will allow for identification of further performance gaps with increased clarity.

Outcome: Two instances of performance issues at the statewide level have already been identified. 1) A slight but persistent decrease in DBS screening rate starting in 2020 was noticed and is now under investigation. 2) A drop in the percentage of timely results reporting by the NBS lab which was due to staffing issues. This is currently improving, and work is underway to implement procedures that prevent such an instance from reoccurring. Using dashboard trends, an in-depth review of collection and transit timeliness for TN facilities is underway and is expected to result in educational outreach and site visits for underperforming facilities.

Conclusion: Utilizing dashboards created with Tableau allows for the visualization of NBS data in a manner such that any user, regardless of data visualization experience, can examine NBS performance trends and identify underperforming areas. The infrastructure necessary for additional dashboards has already been developed with challenges identified and lessons learned from the first dashboard’s creation consequently reducing the burden of developing more dashboards. In addition, survey data was collected to identify how to best visualize data in a dashboard for end users. By taking advantage of these experiences, TN NBS will be able to take a more proactive approach to NBS quality improvement by enhancing or developing new decision-making procedures from dashboard trends and learnings.

Presenter: Charles Lechner, Tennessee Department of Health: Division of Laboratory Services, Charles.Lechner@tn.gov
Second-tier Confirmatory Testing for Hemoglobinopathies in Texas
S. Thompson, J. Lewis, R.C. Lee, S. Tanksley and J.M. Leavitt, Texas Department of State Health Services, Austin, TX

Objective: In February 1995, Texas added molecular genetic testing for the identification of Hemoglobins S, C, and E. The objective of this study is to discuss the confirmatory second-tier testing of hemoglobinopathies and opportunities for the future.

Methodology: DNA testing of hemoglobinopathies is performed on samples that meet the cut-off criteria from the first-tier Hemoglobin screening team. RT-PCR is performed using the ABI StepOnePlus to confirm point mutations that result in Hemoglobin S, C, E, D, O-Arab and two Beta thalassemia mutations, -29 and -88. Automated DNA Sanger sequencing is performed within the amplified region of the Beta globin gene using the ABI 3500xL Genetic Analyzer on specimens absent for both Beta thalassemia mutations, those with unusual allelic ratios in any of the RT-PCR tests, F only specimens, and special cases approved by the medical director. Subsequent sequences are analyzed using SoftGenetics Mutation Surveyor® software and are independently analyzed by two technologists. Any identified variants are interpreted and reported based on HbVar: A database of Human Hemoglobin Variants and Thalassemia mutations as well as utilizing recommendations from the American College of Medical Genetics and Genomics.

Results and Conclusion: Between June 2018 and December 2021, 1445 specimens were DNA tested for hemoglobinopathies with 1356 specimens tested using RT-PCR and 89 specimens reflexed to sequencing. Specimens reflexed to DNA testing are not tested if the patient has a previous Hemoglobin DNA result and the transfusion status is identical to the previously tested specimen. For any RT-PCR tested specimens, the allelic discrimination plot is reviewed to determine if the same hemoglobinopathy result identified by isoelectric focusing and high-performance liquid chromatography is confirmed by DNA testing. If there is a discrepancy between the results, then the specimen will be retested. For specimens reflexed to Sanger sequencing, with the exception of benign mutations, any mutations found are confirmed on both the forward and reverse directions. Subsequent interpretations discussing the potential phenotypic effects are then drafted to report to the submitter. In October 2021, the methodology of sequencing was updated from using the Qiagen gel extraction and spin column kits to the BigDye Direct and Zymo clean-up kits. The ABI 3130 (4-capillary array) Genetic Analyzer was upgraded to the ABI 3500xL (24-capillary array) Genetic Analyzer. This has reduced the hands-on time for testing as well as the run time. Other quality improvements to be implemented include more efficient use of Mutation Surveyor® as well as improving the variant review process.

Presenter: John Leavitt, Texas Department of State Health Services, john.leavitt@dshs.texas.gov
Newborn Screening for Severe Combined Immunodeficiency in Texas Using Multiple of the Median
D. Seidel, K. Collins, R. Lee and S. Tanksley, Texas Department of State Health Services, Austin, TX

Objective: To share the Texas Newborn Screening (NBS) Program’s experience implementing Multiple of the Median (MoM) to calculate T-cell receptor circle (TREC) assay results. We give an overview of our validation study and approach for establishing MoM-based cutoffs and compare assay performance using MoM to our previous method using TREC standards. Lastly, we discuss challenges and considerations for MoM implementation in NBS programs.

Background: TREC quantities were previously calculated using standards with known copy numbers. This method required consistent preparation of standard material and introduces variability when calculating quantities at concentrations near the limit of detection. Along with the implementation of a multiplex assay for Severe Combined Immunodeficiency (SCID) and Spinal Muscular Atrophy in June of 2021, MoM was adopted as an alternative approach to TREC quantification for the detection of SCID. MoM measures how far an individual cycle threshold (Ct) result deviates from the population median Ct.

Methodology: Over 9,000 specimens including SCID-positive patient specimens, quality control materials (Centers for Disease Control; Atlanta, GA), and TREC standards (Perkin Elmer; Waltham, MA) were used in the validation. Dried blood spots were assayed using an automated DNA extraction method, followed by RT-qPCR on a QuantStudio 12k Flex instrument to detect the presence of TREC, SMN1 exon 7, and RNase P. Data was analyzed to establish cutoffs based on the MoM values of extremely low (< 1000g) and normal (≥1000g) birth weight populations. TREC and RNaseP Ct data from one year of screening using the new algorithm was used to evaluate population median and cutoff stability, and positive predictive value of the screen. Results of the analysis were compared against our previous method of TREC quantification.

Conclusion: Using MoM to calculate TREC assay results provides normalized data that avoids variability of TREC standards and improves testing precision. Implementation of MoM requires accurate estimation of the population median, and continuous monitoring of assay performance between reagent lots and preparations is critical for accurate result interpretation.

Presenter: Derek Seidel, Texas Department of State Health Services, derek.seidel@dshs.texas.gov
The Utility of a Five-Spot Punch in Hemoglobinopathy and SCID Testing to Investigate Inconsistent Results and the Educational Outreach

R. Tangalos, D. Seidel, C. Moore, E. Fitch and A. Schlabach, Texas Department of State Health Services, Austin, TX

Objective: Describe the utility of the five-spot punch to investigate atypical newborn screening (NBS) results when multiple sources of blood are suspected and the educational outreach that occurs when adult blood is identified.

Background: Contamination of newborn specimens with adult blood noticeably impacts hemoglobinopathy and SCID screening results. Newborn blood contains a greater proportion of hemoglobin (Hgb) F than Hgb A. By age one, nearly all Hgb F has been replaced by Hgb A. Therefore, screening specimens collected at ≥ 1 year of age for hemoglobinopathies using NBS parameters will yield abnormal results. Similarly, normal newborn SCID results reflect an abundance of T-cell Receptor Excision Circles (TREC) in circulation that decreases with age. Screening of NBS specimens containing adult blood using newborn cutoffs often yields abnormal SCID results. Transfusions can also affect NBS results but are not the only source of adult blood contamination. Since 2014, DSHS has identified 29 specimens that had a mix of adult and infant blood on the card.

Methodology: All specimens with abnormal results receive confirmatory testing if sufficient sample is available. Inconsistencies between testing phases, or between newborn and follow-up screens, can be investigated using the five-spot punch to determine if the specimen contains multiple sources of blood. The five-spot punch procedure includes notation of the spot punched and the specific location of the punch, or punches, from each spot if the spot appears heterogeneous. Hgb results of a correctly collected specimen will show consistent banding patterns across all punches. Different banding patterns, or the presence of adult-only banding patterns, usually indicate multiple sources of blood were collected, and the specimen should be reported out as unsatisfactory. SCID results that are highly inconsistent across punches, or between newborn and follow-up screens, may also indicate similar contamination. When specimens with two sources of blood are identified, DSHS NBS Laboratory education staff reach out to the submitter to alert them to the situation, provide background information, education, and request a recollection.

Conclusion: The five-spot punch procedure enables identification of specimens contaminated by adult blood or blood from multiple sources and reduces the number of incorrect results reported. By providing enhanced communication and education DSHS aims to reduce the collection of contaminated specimens that can delay results.

Presenters: Amy Schlabach, Texas Department of State Health Services, amy.schlabach@dshs.texas.gov and Rebecca Tangalos, Texas Department of State Health Services, rebecca.tangalos@dshs.texas.gov
Liquid Chromatography Tandem Mass Spectrometry for an Ever-Increasing Expansion of Newborn Screening Before or After DNA Sequencing
M. Gelb, M. Campagna and X. Hong, University of Washington, Seattle, WA

Arguably, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the only available technique that can keep up with biochemical newborn screening of an ever-increasing expansion to include new conditions. As more and more DNA sequencing is used in newborn screening it will be required to evaluate DNA results with a biochemical first or second tier analysis (LC-MS/MS). In part 1 of the presentation we will show a number of highly multiplexed LC-MS/MS assays (both initial assay results and results of pilot studies) that cover all of the conditions that have recently been added to the RUSP as well as other conditions that will be considered for RUSP addition in the near future. In part 2, we will provide estimates of the number of newborns identified to be at risk to develop a disease based on first-tier DNA sequencing and show how LC-MS/MS can be used in a second-tier analysis as part of newborn screening to find the newborns that are screen positive (in order to minimize false positives).

Presenter: Michael Gelb, University of Washington, gelb@uw.edu
Considering Screening Newborns for Congenital Cytomegalovirus in Washington State
C. Maloney¹,², C. Ng², M. Katsuyama², J. Thompson²; ¹University of Washington School of Public Health, Seattle, WA, ²Washington State Public Health Laboratories, Shoreline, WA

Background: Congenital cytomegalovirus (CMV) is the most frequent cause of non-genetic hearing loss in children and untreated infections can have detrimental effects on newborn development. The prevalence of congenital CMV disease is higher compared to other conditions on newborn screening panels; however, few programs screen infants for CMV. One state and two Canadian provinces universally screen newborns for CMV at birth while seven states require targeted CMV testing after failed newborn hearing screens. The Washington CMV Project requested the State Board of Health mandate hearing targeted CMV screening. After reviewing available evidence, the Board directed the Department of Health to convene a technical advisory committee in the summer of 2022 to consider screening Washington newborns for CMV.

Objective: The objective is to present our economic evaluation for congenital CMV screening using universal and hearing targeted screening models. We will also report additional findings from the technical advisory committee (meeting to be held in August or September 2022).

Results/Discussion: There are five Board-approved criteria for a technical advisory committee to consider in their evaluation. The fifth criteria, an economic analysis, is a unique policy to Washington State. This presentation will summarize the cost-benefit analysis, discussing the impact and feasibility of screening newborns in Washington for CMV. This analysis will guide discussions on screening benefits and costs during the technical advisory meeting and can be easily adapted by other newborn screening programs to make predictions for outcomes in their populations.

Presenter: Caitlin Maloney, University of Washington, cmm8019@uw.edu
KOH-based Buffer: A Cost-effective DNA Extraction Solution for Dried Blood Spots in SMA/SCID Assay
W. Dansithong, K. Logerquist, R. Hancey, A. Jeffrey, K. Ashment and A. Rohrwasser, Utah State Public Health Laboratory, Taylorsville, UT

Background: A laboratory-developed quantitative PCR test is used for SMA/SCID screening in the Utah Newborn Screening laboratory. In this real-time PCR triplex assay, three target genes are measured simultaneously. TREC and SMN1 are the markers for SCID and SMA, RNASEP serves as an extraction and assay control. Extraction of DNA from dry blood spots (DBS) is performed using the Tecan Evo 200 system and Generation DNA Solution 2 (Qiagen). Potassium hydroxide-based extraction buffer (KOH buffer) has been previously used to extract DNA from DBS. We validated a KOH buffer solution by comparing performance relative to the current method/reagent.

Method: Control dried blood spots (PC-DBS) were punched into 96-well plates in replicates of 5 for a total of 5 days. PC-DBS contains TREC, SMN1 and RNASEP. On each day, duplicate extraction plates were prepared using Generation DNA Solution 2 (Qiagen) and KOH buffer (150 mM Tris, 75 mM KCl, 50 mM KOH). The extracted DNAs obtained from both reagents were transferred into a 384 wells plate containing the triplex master mix and analyzed on the Roche LightCycler 480 II qPCR system. SMA positive DBS and SCID positive DBS were also included in this reagent comparison.

Results: Both reagents resulted in low variability for RNASEP, SMN1, and TREC with similar coefficients of variation (CV). All SMA positive (SMN1 negative) DBS samples, also extracted with both reagents, showed no amplification of the SMN1 gene. Similarly, SCID control material extracted with both reagents did not result in TREC amplification. Control material with low TREC concentrations showed late amplification and resulted in abnormal screening designation for both reagents. RNASEP, the reference gene, presented in the normal range. All SCID screen-positive samples were confirmed using the Enlite TREC (Perkin Elmer) second-tier in-situ assay, quantitatively measuring TREC copy number, confirming low TREC concentration, and indicating concordant screening results.

Conclusion: The KOH buffer performs similarly to the current standard, Generation DNA Solution 2 (Qiagen) and is efficient to use for DNA extraction from DBS material in the SMA/SCID triplex PCR assay. The results from this study establish the equivalency of the KOH buffer for DNA extraction from DBS and highlight this buffer as a cost-effective alternative extraction solution.

Presenter: Warunee Dansithong, Utah State Public Health Laboratory, wdansithong@utah.gov
Improving Timeliness of Diagnosis for Single CFTR Mutation Results through Earlier Notification by NBS Follow-Up

M. Lowe and C. Crews, Virginia Department of Health, Richmond, VA

The Virginia Department of Health’s Newborn Bloodspot Screening Follow-Up program has seven pediatric pulmonary centers around the state for referring presumptive positive and other out of range Cystic Fibrosis CFTR results. An opportunity for a process improvement was identified for the notification of single mutation CFTR results to both the primary care providers (PCPs) and CF specialists for improved communication and timeliness of diagnosis.

Historically, single mutation CFTR results were reported to the follow-up provider of record by the NBS laboratory. The CF specialists were not notified by the NBS program of single CFTR mutation results and relied on the PCP to refer to the CF center or order diagnostic testing. The Follow-Up team would typically request follow-up from the provider at 1 month of life, as part of a bi-monthly open caseload report. Delays in follow-up occurred including desensitization of the need for follow-up by the provider, change in follow-up provider for the infant, and need for education on appropriate follow-up for single mutation CFTR results. Case study reviews have shown a significant delay in follow-up and case closure for single mutation CFTR results.

Implementation of a new initiative for improving communication with the CF specialists by providing notification of single mutation CFTR results by the NBS Follow-Up team within one week of the single CFTR mutation result. This initiative, communicated through REDCap, provided the CF specialists with the knowledge of infants to anticipate referrals and the ability to preemptively reach out to the PCPs in their caseloads if corresponding referrals were not received.

Data will be analyzed and presented showing the time to diagnosis pre and post implementation of this new initiative. The improved process of reporting single CFTR mutation results simultaneously to PCPs and CF specialists will improve timeliness in diagnosis and outcomes.

Presenter: Mary Lowe, Virginia Department of Health, mary.lowe@vdh.virginia.gov
The Virginia Newborn Screening Program has been screening infants for cystic fibrosis (CF) since 2006. Initially, this screen relied solely on a measurement of immunoreactive trypsinogen (IRT), a high level of which can be indicative of CF. In 2011, Virginia’s Division of Consolidated Laboratory Services (DCLS) added a 39-variant Luminex panel as a second-tier assay, creating a more robust screen than IRT alone. In 2021, a retrospective analysis of CF patients in Virginia revealed that the variants present in the state are more numerous and diverse than the composition of the Luminex second-tier panel. This retrospective review incorporated data from a variety of sources, including DCLS’ LIMS system, the CF Foundation’s Patient Registry, and the NBS follow-up team at the Virginia Department of Health. DCLS plans to expand its second-tier screen for CF, moving from the Luminex 39-variant panel to a custom next-generation sequencing assay that will test for over 400 variants classified in the CFTR2 database. This presentation will focus on the retrospective analysis for the current screen and highlight key data points that led to the decision to expand.

**Presenter:** Christian Alcorta, Virginia Division of Consolidated Laboratory Services, christian.alcorta@dgs.virginia.gov
Successful Partnership between NBS and IT Staff in Washington State

L. Christensen¹, R. Sampson¹, E. Rankin¹, J. Rhodes²; ¹Washington Public Health Laboratories, Shoreline, WA, ²Washington Health Technology Solutions, Olympia, WA

The COVID-19 pandemic brought a wave of new funding to the WA State Public Health Laboratory for many things, including IT support. While that funding has been primarily used for pandemic response work, it has also allowed our IT department to increase the staff and capabilities to assist non-COVID work. There are several things our Newborn Screening Program has wanted to implement in the past to increase our efficiency but was unable to due to lack of resources. Now that we have a robust support service, we have been able to kick off two key projects.

Project 1 – Online Card Ordering System. Our IT staff was able to create a portal where all NBS specimen cards, pamphlets, and envelopes can be ordered straight from a website. This reduces staff time for processing phone, fax, and email orders. Our shipping team can go to one location and see all current orders to process. The system also includes a UPS tracking number so that submitters can see where their order is. We are currently working on adding some reporting options that can tally how many cards were sent out during a period of time.

Project 2 – Online Demographic Changes. Our NBS program regularly gets phone calls and emails about small demographic changes that need to be made to a baby’s information. Changes such as dates and times of birth or collection, birthweight and twin status could impact the interpretation of results. With our new demographic changes portal, a submitter can request access through the Secure Access Washington portal to request demographic changes for babies born at their facility. An email is generated that does directly to our laboratory leads to process and re-confirm test results and the requestor receives an email upon completion of the changes in our laboratory information management system.

The partnership between IT and NBS has allowed us to become more effective and efficient with our resources. It also provides better customer service to our partners, empowering them to make and track changes in real time.

Presenter: Leann Christensen, Washington Public Health Laboratories, leann.christensen@doh.wa.gov

P-77 – Withdrawn
Reducing Screening False Positive Rate by Incorporating Additional Steroid Profile Analysis in Newborn Screening for Congenital Adrenal Hyperplasia
M. Berry, M. Loehe, M. Hansen, E. Bialk and M. Baker, Wisconsin State Laboratory of Hygiene, Madison, WI

**Background:** Traditionally, the measurement of 17-hydroxyprogesterone (17OHP) is used in newborn screening (NBS) for congenital adrenal hyperplasia (CAH) caused by 21-hydroxylase deficiency, and high screen false positive rates have been a major challenge for NBS programs. In order to improve NBS for CAH, the Wisconsin NBS laboratory has incorporated an additional testing process since March 1, 2018. Specimens with elevated 17-OHP are reflexed for a quantitative steroid profile analysis that is comprised of 17-hydroxyprogesterone, 11-deoxycortisol, 21-deoxycortisol, androstenedione and cortisol. Concentrations of the steroids are obtained by using a liquid chromatography mass spectrometry assay. Birth-weight associated cutoffs are used in steroid profile analysis.

**Objectives:** To assess the outcomes of additional steroid profile analysis in NBS for CAH, and the need for further refinement of steroid profile components.

**Methods:** Comparison of CAH screening positive predictive values (PPV) between the period of CAH screening by 17-OHP alone (Period One, Mach 1, 2014 to February 28, 2018) and CAH screening by 17-OHP and the steroid profile analysis (Period Two, March 1, 2018 to February 28, 2022).

**Results:** There were 18 confirmed CAH cases in Period One, and 17 confirmed CAH cases in Period Two. During Period One, there were 413 reported screen positive cases, but there were only 41 reported screen positive cases in Period Two. The PPV for CAH screening was 4.4%, and the PPV for CAH screening was 41.5% in Period Two. We further observed that 21-deoxycortisol was elevated in all CAH cases caused by 21-hydroxylase deficiency, while 11-deoxycortisol is an informative analyte for CAH caused by congenital adrenal hyperplasia due to 3-beta-hydroxysteroid dehydrogenase deficiency, a more common form of CAH in Plain communities.

**Conclusions:** Our four-year experience of incorporating steroid profile analysis into NBS for CAH significantly improves the screening false positive rate. Furthermore, knowledge that 21-deoxycortisol is a key analyte in identifying CAH in NBS allows us to make evidence-based consideration to refine the steroid profile, and the inclusion of 11-deoxycortisol is beneficial in in identifying CAH caused by3-beta-hydroxysteroid dehydrogenase deficiency.

**Presenter:** Michelle Berry, Wisconsin State Laboratory of Hygiene, michelle.berry@wisc.edu
Wyoming Newborn Screening Emergency Procedures Plan: Successes and Lessons Learned
C. Soule and E. Dubreus, Wyoming Department of Health, Cheyenne, WY

Maintaining continuity of newborn screening programs during emergency situations is challenging. Babies identified by newborn screening programs are among the most vulnerable in emergency situations because many genetic and metabolic conditions require immediate diagnosis, follow-up, and treatment. Due to the absence of a newborn screening laboratory and follow-up specialists within Wyoming, the Wyoming Department of Health (WDH) partners with Colorado for these services. In light of Wyoming’s rural and frontier nature, the distance of much of our state to the Colorado laboratory and specialists, and weather that can often impact newborn screening sample transportation the Maternal and Child Health Unit (MCH) worked closely with the Public Health Preparedness and Response Unit (PHPR) to develop a continuity of operations plan (COOP). A COOP for the Newborn Screening Program will help ensure that this essential function has the resources and tools needed prior to an emergency or disaster.

In 2020 the WDH was assigned a Public Health Associate through the Centers for Disease Control and Prevention, whose focus was creating a COOP for the Newborn Screening Program. The COOP would be used to guide WDH in the event of any disasters and emergencies at the local or state level that may impact newborn screening operations. MCH and PHPR developed an Emergency Procedures Plan (EPP) to provide the framework to facilitate collaboration among state and federal agencies, local, territorial, tribal and regional efforts to manage any emergency or disaster that could impact the continued and timely collection, transportation, and processing of newborn screening specimens.

This poster will review and detail the successes and lessons learned along the way in creating the COOP, as well as strategies used to engage partners in the process. It will provide a description of the COOP that was created, how it will be exercised, and discuss the tiered decision-tree system.

Presenter: Carleigh Soule, Wyoming Department of Health, carleigh.soule@wyo.gov
Rapid LC-MS/MS First-Tier Newborn Screening Assay with Equivalent Throughput to FIA-MS/MS
S. Isenberg, A. Pickens, C. Cuthbert and K. Petritis, Centers for Disease Control and Prevention, Atlanta, GA

Introduction: First-tier newborn screening evaluates over 40 clinically significant analytes to provide early detection of more than 30 metabolic disorders. Most of these analytes are screened using a multiplex tandem mass spectrometry (FIA-MS/MS) assay with a throughput of approximately two-minutes per sample. We have developed a two-minute LC-MS/MS assay employing a HILIC guard column for separations, to analyze first-tier newborn screening biomarkers with the same throughput as current FIA-MS/MS approaches that do not employ separations. Furthermore, the newly developed two-minute LC-MS/MS assay requires minimal modifications to current instrumentation used in newborn screening labs, only requiring a guard column between the autosampler and mass spectrometer inlet.

Methods: Dried blood spot quality control samples produced in-house were prepared by extracting a 1/8” diameter punch with 100 µL of 80/20 acetonitrile/water containing hydrazine, formic acid, and isotopically-labeled internal standards. The extraction was completed at 45 °C while shaking for 45 min. The eluent was transferred to new wells of a 96-well plate. Analyses were completed on Waters Xevo TQD with an Acquity LC system and on an Agilent Ultivo triple quadrupole mass spectrometer with a 1290 Infinity II LC system.

Results: Adding LC separations to current first-tier newborn screening analyses significantly improves sensitivity by decreasing ionization suppression. For example, citrulline, a first-tier biomarker for citrullinemia, exhibited an increase in absolute signal intensity of a factor of 5 and a corresponding signal-to-noise improvement of an order of magnitude. Additionally, LC separations can separate isobaric biomarkers and interferents. For example, C26-lysophosphatidylcholine (26:0 LPC) is the biomarker for adrenoleukodystrophy (ALD) and exhibits a high reflex rate of up to 3% to second-tier screening due to an endogenous isobaric interferent which is currently resolved by second-tier screening. By introducing chromatographic separation in first-tier screening, 26:0 LPC is resolved from its endogenous interferences, greatly improving the specificity of the assay while reducing or eliminating the need to reflex to second-tier screening. Additionally, hydroxy- and dicarboxy-acylcarnitine pairs such as C3DC and C4OH, which are biomarkers for organic acid disorders, can be resolved by LC separations. Currently, these isobaric pairs can only be distinguished in first-tier newborn screening by adding a derivatization step during sample preparation.

Conclusions: These results demonstrate a simple instrument modification of installing a guard column during first-tier tandem mass spectrometry screening, which improves analytical sensitivity and clinical specificity, all while maintaining the throughput of current FIA-MS/MS first-tier newborn screening assays.

Presenter: Samantha Isenberg, Centers for Disease Control and Prevention, sisenberg@cdc.gov
Measurement of Non-reducing Terminal Glycosaminoglycan Fragment Increases Specificity of Second-Tier Testing for Mucopolysaccharidosis Type I (MPS I)


Mucopolysaccharidosis (MPS) disorders are those that result in a disruption of the catabolism of glycosaminoglycans. MPS I is caused by a deficiency in the enzyme, alpha-L-iduronidase (IDUA). Measurement of IDUA activity by MS/MS is effective in primary screening, however, pseudodeficient alleles will also result in low activity measurement. Thus, more testing is required to reduce false positives. As DNA genotype is often inconclusive the use of targeted sequencing as the second-tier test is unsuitable. A more appropriate approach is to introduce a biochemical test, targeted sequencing would then be implemented as the third-tier.

The biochemical target of second-tier testing is the accumulating species, the glycosaminoglycan. Current methods have been focused on observing elevations in broad classes of glycosaminoglycans. Overall, these assays have been beneficial, however, scientific advancements have opened the door to increasing the power of this testing.

Recently, Dr. Maria Fuller (University of Adelaide) discovered that in MPS affected subjects, an endogenous human enzyme will cleave the non-reducing end of the accumulating glycosaminoglycan. This reaction does not occur to the same extent within the normal population, resulting in the fragment being essentially undetectable. Based on this, measurement of these glycosaminoglycan fragments would allow for specific diagnosis of MPS disease, including subtype.

We sought to validate the measurement of the MPS I marker from dried blood spots in an effort to assess the potential as a second-tier test for MPS disorders in newborn screening. A total of 41 DBS samples were analyzed including apparently normal (25), known MPS I pseudodeficient/carrier (13), MPS I VUS (1), and known MPS I positive (2).

The MPS I marker response was used to calculate a semi-quantitative value that is referred to as the MPS I marker ratio. In both the normal and pseudodeficient/carrier populations, the marker was not typically observed, therefore the ratio was often below the LLOQ (0.14). The normal reference range was defined as three standard deviations from the mean, which resulted in a cutoff of 0.91. All normal samples had marker ratios below this cutoff. The adult MPS I sample had a ratio of 2.43, while the MPS I positive newborn had a ratio of 25.97, well above the cutoff. Only one of the 13 pseudodeficient/carrier samples had a ratio above 0.91. Thus, this assay was found to be 97.7 % accurate with 100 % sensitivity and 97.6 % specificity in identifying a true MPS I positive.

The results of this study support the conclusions drawn by Dr. Maria Fuller and Dr. Michael Gelb. Not only does the measurement of the non-reducing terminal residue increase specificity in comparison to the broad class assays, but it also provides a specific result that can be more impactful in reducing the diagnostic odyssey for these children.

Presenter: Sara Smith, PerkinElmer Genomics, sara.smith@perkinelmer.com